JOURNAL OF DAIRY SCIENCE

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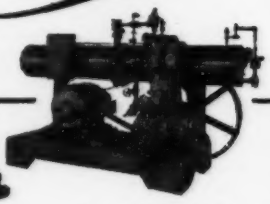

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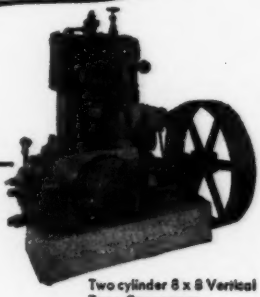

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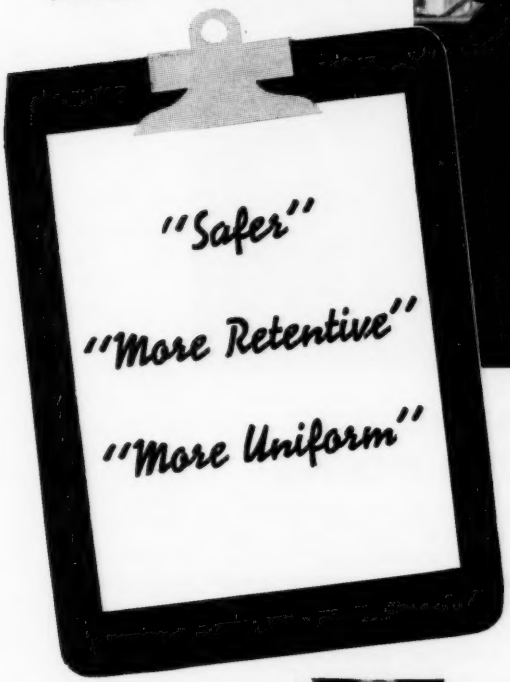

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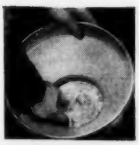

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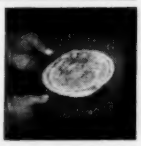

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VOLUME XXXVI SEPTEMBER, 1953 NUMBER 9

PARTICLE SIZE OF DRIED MILK PRODUCTS. I. A STATISTICAL APPROACH TO THE MEASUREMENT OF PARTICLE SIZE [1, 2]

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In studying the particle size of powders it became necessary to devise a suitable sampling technique and to determine how many fields must be measured in order to obtain a representative average value of the complete population.

The literature on particle size of milk powder is rather meager and generally reports an average value based on a specified number of individual measurements (7, 8, 5). The number of individual particles that must be measured so as to yield a value that has significance is an important consideration.

The statistical method used in this study was to measure the variation among slides and fields within individual slides (3, 6).

PROCEDURE

The statistical approach was taken in order to determine (a) whether any significant differences existed between individual slides of the powder, each slide having been prepared using powder obtained from different parts of the same container, and (b) how many fields must be examined and all their particles measured to obtain a mean size value representative of the sample under examination.

Most of the powders examined were obtained in 5-lb. cans. The sampling procedure employed was that recommended by the American Dry Milk Institute (1).

Microscopic examination of milk powder in the dry state was found to be impractical because of excessive clumping of the particles. This difficulty was

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[1] Data presented in this paper were taken from a thesis submitted by the senior author to the Graduate School of The University of Wisconsin in partial fulfillment of the requirements for the Doctor of Philosophy degree, 1952.

[2] Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

overcome by the use of mineral oil mounts as reported by Tracy *et al.* (7) and Hallquist *et al.* (4). The method of Tracy *et al.* was employed as follows: About 0.2 g. of powder was well mixed with 5 ml. of mineral oil. A drop of this suspension was placed on a clean microscope slide and covered with a cover slip. The cover slip was applied in such a manner as to avoid the entrapment of air bubbles.

The slides were examined using the Bausch and Lomb Photomicrographic equipment model L and a Bausch and Lomb CC research microscope. The actual measurement of the particles was made using an ocular disc (Whipple Disc) which had been calibrated against a stage micrometer.

The particle size was recorded in class intervals of the size of the smallest grid division (equal to 7 μ with the magnification employed). The classes used were less than 1 division, 1-2 divisions, 2-3 divisions, and so on up to 30-31 divisions.

It should be pointed out that milk powder particles were not all spherical. In examining samples of powder a variety of particle shapes were encountered, ranging from irregular and elongated to oval and spherical. The spherical ones did not present any problem in measurement of their diameters. Diameters of elongated and oval particles may best be estimated by taking half the sum of their two dimensions. Another approach used here was to take the measurement diagonally at an angle of 45° to the long axis and through the center point of the particle (2).

The experiment for part I, designed to show the effectiveness of the sampling procedure, was set up as follows: Ten random fields for each of four slides were examined for four different powders. Every particle was measured in each field.

The answer to part II was obtained by calculating the semi-confidence limits for 5, 10, 20, and 40 fields using the data accumulated in part I above.

RESULTS AND CALCULATIONS

The mean particle sizes for 10 fields from each of four slides for one powder are presented in Table 1, with the analysis of variance in Table 2. The experiment was repeated using three other powders. The analysis of variance for all four powders is presented in Table 3. The F values between slides for all four powders were nonsignificant, indicating that variations between slides were of the same order as within slides. This leads one to conclude that the method of sampling the powder for the microscopic examination was satisfactory. The chi-square test of homogeneity showed that the mean squares between fields within slides for the four different powders were heterogeneous; accordingly, the data for each powder were treated separately.

The semi-confidence limits calculated from the data in Table 3 and presented in Table 4 show that increasing the number of fields examined decreased the range of the confidence limits, indicating an increase in the accuracy of the sample mean. However, the increased efficiency obtained beyond that found for 10 fields is not warranted because of the extra time and effort involved.

TABLE 1

The mean particle size for individual fields examined on 4 slides of one powder

Fields	Av. value (mean) per field				
	Slides				
	1	2	3	4	
1	2.65	2.75	2.36	3.14	
2	2.29	2.81	2.80	2.63	
3	3.09	2.69	2.79	1.93	
4	2.46	3.19	3.87	1.93	
5	2.71	2.13	2.29	2.74	
6	2.50	1.66	3.71	2.91	
7	3.00	3.11	3.50	3.15	
8	2.69	2.76	2.50	3.96	
9	2.50	2.50	2.53	1.86	
10	2.11	3.02	2.27	3.40	
$\bar{x}$	2.60	2.66	2.86	2.76	2.72

TABLE 2

Analysis of variance

Source of sums of squares	Degrees of freedom	Sums of squares	Mean square or variance	F.
Between slides	3	0.40	0.133	0.45
Between fields within slides	36	10.46	0.291	
Total	39	10.86		

Furthermore, the actual unit of measurement permitted accuracy to the nearest whole unit (7 μ), being limited to this by the disc grid and the magnification used. Any specific recording of particle size as a fraction of the unit of measurement can be only an approximation. The data also indicate that for fine powder, where the variation in size is less, examination of fewer fields was required for the same accuracy when expressed in the units used.

The mean particle sizes for the four powders given in Table 3 are estimates of the true or population means of these powders. The confidence interval $\bar{x} \pm t_{.05}s_{\bar{x}}$, which for powder 1, assuming 10 fields, is 2.72 $\pm$ 0.39 units, gives the range about the sample mean within which can be said with 95 per cent assurance that the true mean of the powder will fall.

TABLE 3

Analysis of variance data for 4 separate powders

Source	D.F.	Mean Square			
		Powders			
		1.	2.	3.	4.
Between slides	3	0.133	0.137	0.017	0.256
Between fields within slides	36	0.291	0.411	0.068	0.146
Mean		2.72	2.84	1.36	2.06

TABLE 4

Semi-confidence limits for different powders

No. of Fields	Semi-confidence limits [a] for different powders			
	1.	2.	3.	4.
5	0.67	0.794	0.325	0.472
10	0.39	0.457	0.181	0.274
20	0.25	0.299	0.121	0.178
40	0.17	0.204	0.081	0.121

[a] Values given in *units*, 1 unit equal to 7 μ.
The degrees of freedom, for calculation of the above data, were 5-1, 10-1, etc.

SUMMARY

A statistical approach to the measurement of particle size has been presented. The measurement of all particles in ten random fields has been shown to be sufficient in obtaining a mean particle size value which is representative of the powder.

ACKNOWLEDGMENT

We are indebted to J. H. Torrie and R. G. D. Steel for their assistance with the statistical analyses.

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REFERENCES

(1) American Dry Milk Institute, Inc. The Grading of Nonfat Dry Milk Solids and Sanitary and Quality Standards. Bull. 911 (Revised), p. 9. 1948.

(2) Dallavalle, J. M. *Micromeritics.* The Technology of Fine Particles. 2nd ed. Pitman Publishing Corp., N. Y. 1948.

(3) Goulden, C. H. *Methods of Statistical Analysis.* John Wiley & Sons, Inc., New York. 1952.

(4) Hallquist, B., Bergerom, R., and Nordh, G. Some Experiences of Airless Spray Dried Milk Powders. XIIth Intern. Dairy Congress II, pp. 94-101. 1949.

(5) Manus, L. J., and Ashworth, U. S. The Keeping Quality, Solubility, and Density of Powdered Whole Milk in Relation to Some Variations in the Manufacturing Process. II. Solubility and Density. J. Dairy Sci., **31**, 11: 935-45. 1948.

(6) Snedecor, G. W. *Statistical Methods.* The Iowa State College Press, Ames. 1950.

(7) Tracy, P. H., Hetrick, J. H., and Krienke, W. A. Effect of Spraying Pressure and Orifice Size on the Physical Characteristics and Keeping Quality of Spray-Dried Whole Milk. J. Dairy Sci., **34**, 6: 583-92. 1951.

(8) Washburn, R. M. The Physical Analysis of Dry Milk. J. Dairy Sci., **5**, 388-98. 1922.

A STUDY OF THE INHERITANCE OF BREEDING EFFICIENCY IN THE BELTSVILLE DAIRY HERD [1]

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AND

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Reliable estimates of the repeatability and the heritability of breeding efficiency are needed so that dairy cattle breeders may determine the effectiveness of selection for fertility, the best selection method to follow, and the relative emphasis to place on fertility and other traits of economic importance.

Lush (8) states that the repeatability of a trait is due to additive genetic merit and the effects of dominance, epistatis, and the permanent environmental factors. Repeatability estimates are useful in predicting the future performance of an individual from records on past performance.

There are two broad causes of phenotypic variability in all characteristics of economic importance; namely, environment and heredity and the interaction between the two. Heritability may be defined as the fraction of phenotypic variance due to differences in additive genetic merit (true transmitting ability) among individuals in a specified population, or as the fraction of the observed variance which was caused by differences in heredity (Lush, 7). Heritability estimates are necessary in determining how best to utilize records on the relatives of an animal, and in obtaining expectations concerning the merit of the progeny of an individual.

Many studies have been conducted on the genetic aspects of breeding efficiency in dairy cattle. Kab (4), Koch (5), Spielman *et al.* (10), Seath *et al.* (9), Clapp (1), Taussig (13), and others have reported considerable variation between the breeding performances of various cow families and daughters of different bulls. These findings indicated that fertility in dairy cattle is controlled to a considerable extent by heredity. Taussig states that fertility is inherited to about the same extent as butterfat production. However, the great chance variation in breeding performance among individuals and small groups, the environmental differences between herds, and environmental time trends could have accounted for much of the variation observed in these studies.

Most studies in which attempts have been made to obtain quantitative measures of the portion of observed variance in breeding performance that is

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[1] These data were made available through a cooperative project between the University of Maryland and the Bureau of Dairy Industry and are taken from a thesis submitted by the senior author for the degree of Doctor of Philosophy in Animal Breeding at Cornell University.

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due to genetic differences support the view that repeatability and heritability values of breeding efficiency are low.

Based on 2,610 records of breeding service from the Maine Artificial Breeding Association, and using 150-180 day non-returns as an indication of conception, Leonard (6) estimates the repeatability of breeding efficiency as 0.075. Dunbar (2) studied records from approximately 1,000 cows in the New York Artificial Breeding Association. Using 60-90 day non-returns to first service as the measure of breeding efficiency, he estimated repeatability as 0.027, and heritability as 0.004.

Tabler *et al.* (11) in a recent study of the breeding efficiency of 19 cow families involving 410 cows found that when the number of days from the first service to conception and the number of services for conception were used as measures of fertility, variation was greater within families than between families.

Tanabe *et al.* (12) found no significant difference in the fertilization rates between 34 cows with four to six previous services per conception and 25 cows with seven to 13 previous services per conception. Trimberger *et al.* (14) found that it was not possible to predict the number of services required by an individual cow for subsequent conceptions from that animal's breeding efficiency during previous years.

This investigation was made to study the environmental factors affecting the reproductive performance of the cows in the Jersey and Holstein herds maintained by the Bureau of Dairy Industry, Beltsville, Maryland, and to obtain unbiased estimates of the repeatability and heritability of breeding efficiency in these herds.

DATA AND PROCEDURE

Data, available on 834 cows, covered the period from 1920 through 1950. During this 30-year period, the Beltsville Holstein and Jersey herds were devoted primarily to longtime breeding projects. These projects generally involved the use of a succession of unrelated proved sires. An effort was made to keep the management of these herds as uniform as possible. Normally all female offspring were maintained until they died or were disposed of because of disease, breeding difficulties, or extreme old age. Exceptions to this practice were several reductions in cow numbers resulting from budgetary limitations.

Three measures of breeding efficiency were selected for study, namely, *(a)* the number of services required for conception, *(b)* the number of days from first service to conception, and *(c)* the regularity of the occurrence of estrus. After tabulation of the data from individual breeding histories, all pertinent information concerning each reproductive cycle was punched on IBM cards. Inspection of the data revealed a slight trend of improved breeding efficiency in the spring and fall months along with fluctuations up and down in breeding efficiency through the years. Because of the observed seasonal trends, the months of the year were grouped into the three consecutive segments, or seasons, that gave maximum mean differences in the three measures of breeding efficiency employed.

The following linear mathematical model was assumed:

$$y_{ij} = \mu + a_i + c_j + e_{ij}$$

y_{ij} is the breeding efficiency as measured in this study, of the j^{th} cow in the i^{th} year-season; μ is the mean of all breeding efficiency records; a_i is the effect of the i^{th} year-season; c_j is the effect of the j^{th} cow; and e_{ij} is the error associated with the breeding efficiency of the j^{th} cow in the i^{th} year-season; a_i, c_j, and e_{ij} were assumed to be independent random variables with variance σ_a^2, σ_c^2, and σ_e^2 respectively.

Estimates of the variance components for year-season, cow, and error were obtained by Method 1 as described by Henderson (3). This method is adapted to obtaining unbiased estimates in nonorthogonal data. Estimates of repeatability then were obtained from the ratio of cow variance to cow variance plus error variance. These data on the Jersey and Holstein herds were analyzed separately to obtain estimates of repeatability and heritability for each breed. Since, other factors being equal, the accuracy of the estimate is increased by increasing sample size, the breed estimates then were combined to obtain estimates for the entire population of 834 cows.

Lush (7) cited the advantages of estimating heritability in much animal husbandry data by computing the regression of offspring on dam within groups of offspring by the same sire. In this investigation intra-breed regression of daughter on dam rather than intra-sire regression was used in estimating heritability. This procedure was followed because the effects of time trends in environment were accounted for in the estimates of the year-season variance component and because mating was considered random because of the system of breeding followed.

Since the expected daughter-dam cross-product value contains one-half of the additive genetic variance of the daughter plus any similarity between daughter and dam due to the observation on both animals occurring in the same year-season, appropriate coefficients for year-season variance components in the cross-product values were also determined.

RESULTS

Repeatability estimates. Using the values of the coefficients of cow variance and error variance secured from the records of all reproductive cycles, the repeatability of breeding efficiency was estimated to be 0.18 from the data on the regularity of the occurrence of estrus, 0.12 from the data on the number of services required for conception, and 0.11 from the data on the number of days from first service to conception. An upward bias is reflected in the repeatability estimate based on the regularity of estrus. This bias was unintentionally created by the manner in which the regularity of the occurrence of estrus was scored. All reproductive cycles for each cow were scored in succession. Since this scoring was conducted over a period of several months, it is probable that there was a tendency to create some similarity between the observations on any one cow. A more objective score might have been determined by scoring only one

cycle of each cow at a time through the entire herd and then repeating the sequence until all cycles of all cows had been scored. However, since cross-products between the daughter and dam records were used in obtaining estimates of heritability, this bias affects the repeatability value only.

These repeatability estimates were obtained on an intra-herd basis and are corrected for variation due to seasonal effects, time trends, and breed differences. However, they are uncorrected for any variation due to age effects since means of the three measures of breeding efficiency obtained for the various age groups did not show any definite trend in breeding efficiency associated with increasing age through 11 years.

As the general practice was to use sires independent of their average fertility, and of their relationship to any other animal in the herd, sire effects were assumed to be random and independently distributed.

Heritability estimates. Using daughter-dam cross-product values derived from the same data employed for the repeatability estimates, and making appropriate corrections for daughter and dam observations that occurred in identical year-seasons, estimates of variance due to the additive genetic merit of the cows were secured. With the values for additive genetic, cow, and error components of variance, it was possible to secure estimates of the heritability of breeding efficiency, since an estimate of heritability may be obtained from the ratio of genetic variance to total variance. The heritability values obtained are 0.05 based on the regularity of estrus, 0.07 based on the number of services required for conception, and 0.07 based on the days from first service to conception. These heritability values were determined on an intra-herd basis and were corrected for seasonal effects, time trends, and breed differences.

TABLE 1

Daughter-dam lifetime comparisons on mean number of services

Lifetime mean No. of services required by dams	Lifetime mean No. of services required by daughters	No. of daughters
1.00 — 1.50	2.41	152
1.51 — 2.00	2.56	156
2.01 — 2.50	2.57	188
2.51 — 3.50	2.61	203
3.51 — 4.50	2.95	87
Over 4.50	3.11	48

In Table 1 the records of all daughters used in obtaining the heritability values are grouped according to the lifetime mean number of services per conception required by their dams. From this comparison it is apparent that the similarity in daughter and dam performance, based on this phenotypic measure of breeding efficiency, is not great. These mean comparisons agree with the low heritability estimates obtained.

DISCUSSION

Unless a herd is devoted to a scientifically controlled investigation of reproductive performance, breeding records maintained on any dairy herd are a by-

product. In no case can these herd records be considered complete lifetime histories of the reproductive efficiency of dairy cows with all cows subjected to uniform breeding conditions and examinations. Such data would be highly desirable for an investigation of this nature. However, much information needed to assist dairymen in planning the most efficient breeding program can be obtained from available records, provided the inadequacies of such records are given due consideration.

Advantages of data. There are certain advantages of the data used for this study. Most of the cows were maintained in the herd until they died or were disposed of for disease, extreme old age, or breeding difficulties. This was a definite advantage because a more complete lifetime picture of the breeding performance of the cows in the herd was obtained than would be possible in a herd where culling for low production was the general practice. Also, the breeding records through the years were more complete than the records maintained in most herds.

A simple comparison of the means of the breeding efficiency measures for the daughter and dam populations of the two breeds is shown in Table 2. This

TABLE 2

Comparisons of daughter and dam means

	No. of reproductive cycles	Mean score on regularity of occurrence of estrus [a]	Mean No. of services required for conception	Mean days from first service to conception
Jersey daughters	1419	0.78	2.62	49.9
Jersey dams	1082	0.75	2.62	50.1
Holstein daughters	1426	0.72	2.48	51.5
Holstein dams	1077	0.70	2.44	48.4

[a] regular = 0, slightly irregular = 1, irregular = 2, and very irregular = 3.

comparison indicates that there was very little, if any, effective selection for breeding performance among the dams.

The herd breeding and management programs were held more constant through changing economic conditions than would be possible in the average breeder's herd. Because of such factors as the weather, disease, and changes in personnel, the herd environment can by no means be considered constant; however, a definite effort was exerted to maintain the feeding, housing, breeding, and management conditions as uniform as possible. Therefore, the variable environmental effects tended to be random rather than the result of intentional differences in feeding and management. This should help to minimize any bias in the estimates of repeatability and heritability as, in the estimation of the variance components, it was assumed that herd environmental changes were independently distributed.

Since the general practice was to use production-tested sires, the majority of the services in the herd were by sires 7 years old or older. This general practice of using relatively old sires, a number of which had average to low fertility, probably resulted in the detection of cows with low fertility more readily than

would have been the case had these cows been bred to bulls of higher fertility. This factor would have a tendency to increase the size of the heritability estimates.

Limitations of data. There are also certain limitations of the data used for this study. Since all cows were registered, were in the same herd, and, in general, were high milk and butterfat producers, they cannot be considered a random sample of the dairy cow population. Therefore, the heritability and repeatability estimates are applicable only to this particular cow population. However, since most breeders do not have either sufficient data or adequate facilities for obtaining values for their own herds, these estimates may be useful guides in other herds.

In this investigation in so far as was known, most of the sources of variation were taken into account. However, there may have been some unknown sources of variation which we failed to account for, and which may bias slightly the estimates which were obtained.

Since two of the measures of breeding efficiency employed were applicable only to cows that conceived, heifers that never conceived were not included in the data. In these herds 3.6 per cent of all virgin heifers bred never became pregnant. The degree of bias introduced into the estimates of heritability by the omission of these "sterile'" heifers depends upon the extent to which this condition in heifers is affected by genetic differences among cows.

Environmental effects. The season of the year has been reported (1, 6, 9) as the cause of considerable variation in breeding efficiency, particularly as measured by the number of services required for conception. In this investigation the months of the year were grouped in such a way as to maximize the variation due to seasonal effects. However, the year-season component accounted for zero per cent of the total variance in the data on regularity of estrus, 4 per cent in the data on the number of services required for conception, and 3 per cent in the data on the days from first service to conception. These values indicate that the effects of season and time trends on breeding efficiency in this herd are not very important. This is in agreement with the findings of Dunbar (2), who obtained unbiased estimates of year-season components of variance from data on artificially sired cows in New York state. Part of the rather large seasonal effects reported by some investigators is apparently caused by small sample numbers and by the fact that in many herds cows are intentionally bred to freshen at some particular season of the year.

The error component of variance amounted to 83 per cent of the total variance in the data on the regularity of estrus, 84 per cent in the data on the number of services required for conception, and 87 per cent in the data on the days from first service to conception. These errors are caused by all of the environmental factors affecting breeding efficiency that were not included in the mathematical model; i.e. by all factors except cow and year-season. A few of these factors are possible age and sire effects, and possible differences in the stage of estrus at which the cows were bred, in the time intervals from parturition to first breeding, in exposures to certain diseases, and in the accuracy with which breeding records were maintained.

CONCLUSIONS

The results of this investigation indicate that the intraherd repeatability and heritability values of breeding efficiency in dairy cattle are low. When the number of services required for conception, the number of days from first service to conception, or the regularity of the occurrence of estrus are used as measures of breeding efficiency, selection for improvement in breeding efficiency will not be very effective. Dairy cattle breeders can make greater progress in the improvement of the genetic merit of their herds by practicing selection for other traits of economic importance, such as high milk production, which has much higher repeatability and heritability values.

Environmental factors that appear to be of relatively minor importance as the causes of variation in breeding performance are age of the cow, season of the year, and time trends. A large body of accurate and complete data, based on a herd where adequate controls are maintained purely for the purpose of investigating breeding performance, is needed in order to obtain a more exact picture of the relative importance of these environmental factors.

REFERENCES

(1) Clapp, E. E., Jr. An Analysis of the Reproductive History of the Cornell Dairy Herd. Master of Science Thesis. Cornell University Library. 1946.

(2) Dunbar, R. S., Jr. Heritability of Fertility in Dairy Cattle. Master of Science Thesis. Cornell University Library. 1950.

(3) Henderson, C. R. Estimation of General, Specific, and Maternal Combining Abilities in Crosses Among Inbred Lines of Swine. Doctor of Philosophy Thesis. Iowa State College Library. 1948.

(4) Kab, E. Untersuchungen über die Vererbung von Fruchtbarkeit und Unfruchtbarkeit beim gelben Frankenvieh (Studies on the Inheritance of Fertility and Sterility in the Yellow Franconian Cattle). Animal Breeding Abstr., 6: 200. 1938.

(5) Koch, W. Vererbung und Unfruchtbarkeit (Inheritance and Sterility). Animal Breeding Abstr., 7: 310-311. 1939.

(6) Leonard, H. A. An Analysis of Factors Affecting Artificial Breeding Efficiency of Dairy Cattle in Maine. Master of Science Research Report. Cornell University Library. 1950.

(7) Lush, Jay L. Intra-Sire Correlations or Regressions of Offspring on Dam as a Method of Estimating Heritability of Characteristics. Proc. Am. Soc. Animal Production, 32: 293-301. 1940.

(8) Lush, Jay L. *Animal Breeding Plans.* The Collegiate Press, Inc., Ames, Iowa. 1945.

(9) Seath, D. M., and Staples, C. H. Some Factors Influencing the Reproductive Efficiency of Louisiana Herds. J. Dairy Sci., 24: 510. 1941.

(10) Spielman, Arless, and Jones, I. B. The Reproductive Efficiency of Dairy Cattle. J. Dairy Sci., 22: 329-334. 1939.

(11) Tabler, Kenneth A., Tyler, W. J., and Hyatt, George, Jr. Type, Body Size and Breeding Efficiency of Ayrshire Cow Families. J. Dairy Sci., 34: 95-105. 1951.

(12) Tanabe, T. Y., and Casida, L. E. The Nature of Reproductive Failures in Cows of Low Fertility. J. Dairy Sci., 32: 237-246. 1949.

(13) Taussig, S. The Problem of Fertility in Dairy Cattle and Its Improvement Through Breeding. Intern. Rev. Agr., 37: 256T-261T. 1946.

(14) Trimberger, George W., and Davis, H. P. Predictability of Breeding Efficiency in Dairy Cattle from their Previous Conception Rate and from their Heredity. J. Dairy Sci., 28: 659-669. 1945.

A SEMI-AUTOMATIC CRYOSCOPE FOR DETERMINING THE FREEZING POINT OF MILK

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The cryoscope described by Hortvet (5) in 1921 has greatly facilitated the routine determination of the freezing point of milk, but this apparatus has certain limitations. As Temple (7) pointed out, the procedure is tedious and it involves the use of ether, which constitutes a hazard. Temple has designed an apparatus which eliminates the use of ether and provides automatic control of the temperature in the cooling medium. However, with his method it is still necessary to stir the sample and tap the thermometer manually. Since these two steps are the most difficult to standardize and the most tedious, it was felt that mechanical methods would insure more uniformity and be more convenient.

The cryoscope described in this paper minimizes individual variations in the interpretation of the directions and in the performance of the determination while retaining the essential features of the Hortvet cryoscope. The ease of operation should encourage more frequent use of the cryoscope and thereby facilitate the study of freezing-point variations. A need for such a study is indicated by the data of Paley and Tzall (6) and Dahlberg, Adams, and Held (2).

APPARATUS AND PROCEDURE

The freezing tube and jacket and the thermometers were made according to the specifications of the Association of Official Agricultural Chemists (1). To provide more accurate temperature control of the cooling medium, a refrigerated bath was designed. Mechanical devices were designed to stir the sample and tap the thermometer. The general construction is shown in Figure 1.

The cryoscope chamber consists of two Pyrex glass cylinders, one inside the other; the inner one is 8¾ in. in diameter and 10 in. high; the outer one is 10⅛ in. in diameter by 10 in. in height. The inner cylinder sits on a rubber ring which provides an air space between the two cylinders and absorbs some of the vibrations. Anhydrous $CaSO_4$ is placed at the bottom of the space between the cylinders to absorb any moisture. The space between the top of the cylinders is sealed off to provide an insulating air space by wedging in a ring made from rubber tubing. The top of the chamber is covered with a ¼ in. bakelite disk. Holes are drilled in the cover to permit the insertion of the freezing tube and jacket, the bath thermometer and agitator. The agitator is an 8-in. solid monel metal stirring rod with a 2-in. propeller which is driven at 800 r.p.m. by a small electric motor.

The refrigeration system consists of a small Freon compressor connected by a flexible plastic tubing to an evaporator coil, made from a 15-ft. length of ⅜-in. copper tube, which is wound in a seven turn spiral 7¾ in. in diameter. The

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tubing is painted with an acid-seal paint to prevent corrosion. The inlet and outlet tubes for the evaporation coil are fitted into slots cut in the cover. The cooling medium is a glycerol-water solution which freezes at —3.0° C. Thymol is used in the solution to prevent bacterial growth. A constant temperature is obtained by maintaining a thin ice bank during operation. Too thick an ice bank must be avoided to eliminate the danger of cracking the cylinder.

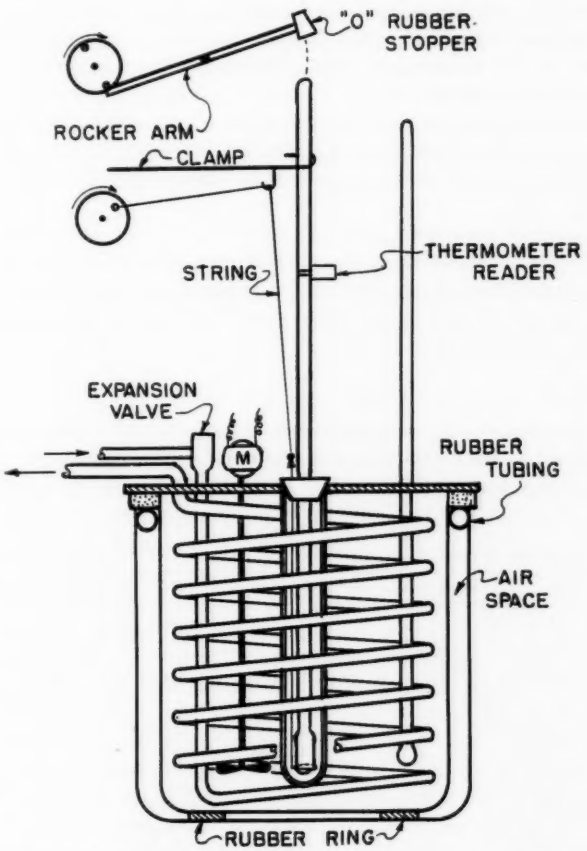

FIG. 1. General construction of the semi-automatic cryoscope.

The agitator for the samples is manipulated by attaching one end of a string to the top of the conventional stirring rod and the other end to a peg on a 3½-in. pulley which rotates at 78 r.p.m. The diameter of the pulley was chosen so that the length of stroke would equal the height of the sample in the freezing tube. The thermometer is tapped by allowing a rubber stopper mounted on a rocker arm to strike the top of the thermometer. The rocker arm pivoting on a metal

rod is raised by means of two pegs on a 3½-in. pulley rotating at 50 r.p.m. By using two pegs the thermometer is tapped at the rate of 100 strokes per minute. When a peg disengages from the rocker arm, the arm falls freely so that there is no positive force applied to the thermometer. The thermometer is tapped with a vertical stroke so as to minimize any horizontal vibration. To minimize parallax error and to get greater magnification a Parr thermometer reader [1] is used in place of the conventional magnifying glass. A 12-in. piece of copper wire is used for seeding instead of the conventional metal tube. The wire is kept in a thermos bottle filled with dry ice and taken out and inserted into the sample tube at the appropriate time, whereupon seeding occurs immediately and the wire is withdrawn.

Since the apparatus is glass, it is possible to maintain the ethyl alcohol in the freezing tube jacket at the same level as the sample in the freezing tube. In the Hortvet apparatus it is practically impossible to ensure a uniform alcohol level.

The procedure prescribed by the A.O.A.C. (1) is followed except as indicated here. The bath and sample agitators are left on continuously until the sample is seeded. The sample is stirred manually as it approaches the freezing point. The tapping device then is turned on and tapping is continued until the freezing point has been determined.

RESULTS AND DISCUSSION

A series of experiments was conducted to compare the results obtained by the modified cryoscope and the Hortvet cryoscope. The same thermometers and sample tube were used in both cases. The results shown in Table 1 illustrate

TABLE 1

Comparison of results obtained with the Hortvet and modified cryoscopes

Sample designation	Hortvet cryoscope [a]			Modified cryoscope [b]		
	Av. observed freezing point	No. of determinations	Max. deviation from mean	Av. observed freezing point	No. of determinations	Max. deviation from mean
	(° C.)		(° C.)	(° C.)		(° C.)
98 A	—0.529	2	0.001	—0.530	2	0.000
167 A	—0.536	2	0.002	—0.539	3	0.001
Raw C.U.	—0.524	3	0.001	—0.525	3	0.001
BBG	—0.545	3	0.001	—0.545	3	0.001
Past. C.U.	—0.529	3	0.000	—0.529	3	0.000
BB	—0.552	3	0.001	—0.553	3	0.001
Lu	—0.533	3	0.001	—0.533	3	0.001
BB-2	—0.526	3	0.001	—0.526	3	0.000
Comp	—0.537	3	0.003	—0.537	3	0.001
5/27 A	—0.533	2	0.001	—0.533	2	0.001
142 A	—0.532	2	0.001	—0.530	2	0.000
156 A	—0.533	2	0.001	—0.535	2	0.001
Av.	0.5340		0.0012	0.5346		0.0007

[a] Thermometer tapped manually.
[b] Thermometer tapped mechanically.

[1] Obtained from Burrell Corporation, catalog No. 10-090.

that the two instruments were comparable. Furthermore, the maximum deviation from the mean is less with the modified cryoscope.

Another experiment was conducted to determine whether or not different operators would get the same results on the two cryoscopes and whether the manner of tapping had any effect on the results. In each case the senior author prepared the samples and carried out the procedure as far as the step where the sample was seeded. Then the different operators took over and completed the determination according to their interpretation of the directions. The first phase of this experiment was designed to compare the two cryoscopes when the official directions were used. These directions read as follows: "Manipulate stirrer slowly and carefully two or three times while the Hg column approaches its highest point. By means of suitable light-weight cork mallet tap upper end of thermometer cautiously a number of times until top of Hg column remains stationary at least 1 minute." The second phase of this experiment was designed to determine whether mechanical tapping and more precise directions would reduce the variations. For this phase the following directions were issued: ". . . As the mercury approaches its highest point (i.e. when the thermometer reads about 0.07° below the expected freezing point) manipulate the stirrer *slowly* and *carefully* 2 or 3 times. Turn on the tapping device as soon as stirring is complete and continue tapping until the top of the Hg column remains stationary for at least 1 minute. . . ." Each operator had made freezing-point determinations previously. They were not allowed to watch each other perform the determination, nor did they know the values obtained by the other members. The same thermometers and sample tube were used in both cryoscopes.

These results, which are shown in Table 2, show definite variations between individuals when manual tapping and the official directions were used. The variations can be attributed principally to the differences in the manner of tapping and/or "last minute" stirring, since the rest of the procedure was carried out by the same person. Except with observer 8, the stirring procedure was very similar—stirring being discontinued about 0.05° C. below the observed freezing point. However, there were considerable variations in the tapping procedure. The fact that the deviations were similar with both cryoscopes indicates that they are the operator's variations and supports the conclusion that the two cryoscopes are comparable.

From the results obtained by manual tapping it is obvious that the height the mercury column attains and the rate of ascent depend to some extent on the vigor and frequency of tapping. It appears that more consistent results are obtained by tapping continuously, whereas irregular tapping tends to give more erratic results. This is confirmed by the fact that mechanical tapping gives consistent results.

The effect of variations in the tapping procedure indicates a definite limitation of the official method and clearly indicates that the operator must calibrate his own thermometer and not depend on another person's calibration. Furthermore, it is questionable whether an individual would be consistent in his tapping procedure, particularly if he is not regularly making determinations. By using mechanical tapping this variable is eliminated.

The effect of stirring too long is indicated by the results of observer 8. Although this in itself is insufficient evidence, the senior author has observed similar results if stirring was not stopped 0.04 to 0.05° C. below the freezing point. In some cases there was no effect, but when it did affect the results the observed freezing point was always lower. In the interests of uniformity it seems advisable to define more precisely the time of this "last minute" stirring. The phrase "as it approaches its highest point" is indefinite; therefore, it is suggested that the following qualifying statement be added: "(i.e. when the thermometer reads about 0.07° below the expected freezing point)."

TABLE 2

The effect of different cryoscopic procedures on observers' variations

Observer designation	Manual tapping				Remarks	Mechanical tapping	
	Hortvet apparatus		Modified apparatus			Modified apparatus	
	Deviation from av.[a]	Time[b]	Deviation from av.[a]	Time[b]		Deviation from av.[a]	Time[b]
	(° C.)	*(Sec.)*	*(° C.)*	*(Sec.)*		*(° C.)*	*(Sec.)*
1	+0.005	100	+0.004	120	Tapped sharply and	+0.001	100
	+0.004	100	+0.003	120	frequently	+0.001	110
2	+0.002	75	+0.003	90	Tapped firmly and	+0.001	100
	+0.002	70	+0.004	90	continuously	+0.001	110
3	.....	.....	+0.002	110	Tapped lightly and	−0.002	100
			+0.002	105	continuously	−0.002	110
4	+0.001	150	+0.003	170	Tapped lightly and	+0.001	110
	+0.003	140	−0.002	150	irregularly	0.000	90
5	+0.004	190	−0.001	140	Tapped lightly and	+0.001	90
	+0.002	100	0.000	180	irregularly	−0.001	105
6	0.000	90	−0.001	120	Tapped lightly and	.....	.....
	0.000	90	−0.001	120	continuously		
7	0.000	160	−0.001	90	Tapped side of ther-	0.000	110
	−0.009	160	+0.001	180	mometer, irregularly	+0.001	100
8	−0.007	150	−0.006	150	Stirred and tapped	0.000	90
	−0.006	160	−0.007	120	alternately	−0.002	80
Av.	±0.0032	124	±0.0026	128		±0.0010	100
Range	0.014	120	0.011	90		0.003	30

[a] The average observed freezing point of all individuals.
[b] Interval from time of seeding until the mercury reached its highest point.

The results (Table 2) showing the rate of rise of the mercury column have interesting aspects. First of all, the fact that the rate with manual tapping was the same for both cryoscopes would indicate that the rate of heat transfer was practically the same. This fact indicates that the differences in the conductivity of the ether and the water-glycerol bath do not have any appreciable effect on the rate of heat transfer. The effect of differences in the rate of heat transfer is discussed in detail by Elsdon and Walker (3). Secondly, the fact that the rate was faster when tapping (either manual or mechanical) was continuous

indicates that the "thermometer lag" is reduced. This reduction in time has the advantage of minimizing the error due to the formation of ice and consequent lowering of the freezing point. Harper (4) has published results which show the importance of "thermometer lag" in temperature determinations.

A collaborative study conducted by nine laboratories in New York and New Jersey reveals additional evidence of the variations in freezing-point values obtained by different individuals. Each laboratory used its own equipment and standardized its own thermometer according to the official method. Tables 3 and 4 show some of the variations. The wide range of values emphasizes a need for more careful study of the method to determine what factors cause these variations. In view of the data presented in Table 2, one might expect that the manner of tapping was one of the factors.

TABLE 3

Deviation of the average of each laboratory from the average of the freezing-point values reported by all laboratories[a]

(Deviations in thousandths of degrees centigrade)

Series No.	No. of samples	Laboratory designations								
		A	B	C	D	E	F	G	H	I
1	4	—12.5	— 3.0	— 3.5	— 4.0	+ 1.5	+ 4.0	+ 6.0	+ 5.0	+ 5.0
2	7	—13.0	— 6.0	+ 2.0	+ 2.5	— 5.0	+ 3.0	+ 1.0	+ 4.0	+14.5
3	7	—15.0	— 3.5	—11.0	+ 1.0	+ 1.0	+ 2.0	.....	+ 7.5	+12.0
4	6	.....	— 6.5	— 1.0	.....	+ 6.5	— 3.5	.....	....	+ 4.5
Av.		—13.5	— 4.8	— 3.4	— 0.2	+ 1.0	+ 1.4	+ 3.5	+ 5.5	+ 9.0

[a] All laboratories followed the official method.

The cryoscope described in this paper was designed to overcome certain limitations of the Hortvet cryoscope without eliminating the fundamental features. Using this modified apparatus one is assured of more uniformity in stirring and tapping and in temperature control. Furthermore, the procedure is easier and faster. A determination can be made in less than half the time required to make a Hortvet determination. The apparatus is constructed of material that either can be purchased from commercial stocks or can be made easily. An all glass chamber is used to permit visual observation of the working parts. Although the use of glass introduces a breakage hazard, it is felt that with reasonable handling this is not a serious objection.

SUMMARY

A semi-automatic cryoscope has been designed which gives results comparable to the Hortvet apparatus. Determinations can be made more accurately and rapidly and with greater ease. The cooling bath temperature is maintained constant by mechanical refrigeration. A glycerol-water solution is used instead of ether as the cooling medium. The sample is stirred and the thermometer is tapped by mechanical means. This eliminates the two most tedious steps and provides more uniformity. The sample is seeded by a copper wire, which is kept in a thermos bottle containing dry ice.

TABLE 4

Freezing points for diluted milk as observed by nine different laboratories [a]

Labs.	A	B	C	D	E	F	G	H	I	Av.	Calc.[b]
% added water	(° C.)	(° C.)	(° C.)	(° C.)	(° C.)	(° C.)	(° C.)	(° C.)	(° C.)	(° C.)	(° C.)
0	—0.555	—0.551	—0.546	—0.551	—0.551	—0.546	—0.553	—0.545	—0.535	—0.5489	—0.5530
1	—0.565	—0.547	—0.543	—0.541	—0.547	—0.539	—0.540	—0.535	—0.535	—0.5425	—0.5445
2	—0.547	—0.541	—0.534	—0.541	—0.540	—0.533	—0.531	—0.530	—0.517	—0.5350	—0.5390
3	—0.537	—0.537	—0.529	—0.522	—0.532	—0.527	—0.525	—0.525	—0.514	—0.5275	—0.5335
4	—0.547	—0.534	—0.522	—0.531	—0.538	—0.523	—0.524	—0.524	—0.511	—0.5305	—0.5280
5	—0.536	—0.527	—0.514	—0.512	—0.523	—0.513	—0.511	—0.512	—0.503	—0.5170	—0.5225
6	—0.516	—0.517	—0.506	—0.494	—0.514	—0.509	—0.518	—0.510	—0.493	—0.5100	—0.5170

[a] All laboratories used the official method.
[b] Calculated values based on Winter's table. (1).

The freezing-point values reported for a sample of milk by eight individuals in our laboratory using the official method of analysis revealed a maximum variation of 0.014° C. The same individuals using the modified method reported freezing-point values with a maximum variation of only 0.003° C. The results of nine collaborating laboratories, using the official method, revealed a maximum variation of 0.0275° C. between the average freezing-point values reported for one series of samples.

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REFERENCES

(1) Association of Official Agricultural Chemists. *Official and Tentative Methods of Analysis.* 7th ed., pp. 235-239 and Table No. 41.28. Washington, D. C. 1950.

(2) Dahlberg, A. C., Adams, H. S., and Held, M. E. Sanitary Milk Control and Its Relationship to the Sanitary, Nutritive and Other Qualities of Milk. National Research Council Publication, No. 250. 1953.

(3) Elsdon, G. D., and Walker, G. H. *Richmond's Dairy Chemistry.* 4th ed. J. P. Lippincott Co., Philadelphia, London, and Montreal. 1942.

(4) Harper, D. R. Thermometric Lag. Bull. Bur. of Standards, **8**: 659-714. 1912.

(5) Hortvet, J. The Cryoscopy of Milk. J. Ind. Eng. Chem., **13**: 198-208. 1921.

(6) Paley, C., and Tzall, B. An Inquiry into the Interpretation of the Freezing Points of Milk Obtained by the Hortvet Cryoscope and Its Relationship to Present-Day Market Fluid Milk. 24th Ann. Rpt. of N. Y. State Assoc. of Milk Sanitarians. 1950.

(7) Temple, P. L. A New Apparatus for the Rapid and Economical Determination of the Freezing Point of Milk. The Analyst, **62**: 709-712. 1937.

VARIATIONS IN THE FREEZING POINTS OF COW'S MILK

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The official method of determining whether or not cow's milk has been adulterated by the addition of water is based on an average freezing-point value of —0.550° C. The value was taken from Winter's table (9) published in 1914. However, the official method of determining the freezing point was not developed until 1921 by Hortvet (11). He obtained an average of —0.548° C. for 75 samples with a range from —0.534 to —0.562° C. In a collaborative study, Bailey (4) in 1922 reported an average of —0.545° C. for individual cows with a range from —0.523 to —0.580° C. All of these samples were collected between March 29 and October 26. Stubbs and Elsdon (18) in 1934 obtained an average of —0.544° C. for 1,000 samples with a range from —0.529 to —0.563° C. Lampert (14) in 1939 reported an average of —0.536° C. for 20 samples of herd milk with a range from —0.529 to —0.547° C. Krienke and Arrington (13) in 1949 obtained results which indicated that the average freezing point was higher than —0.550° C. They also observed differences in the average freezing points for Jersey and Holstein milk. Paley and Tzall (16) in 1950 presented data which show an average (computed from their graph) of —0.532° C. for 1,372 samples of market milk, of which only 0.65 per cent had freezing points as low as —0.550° C. and 33 per cent had freezing points between —0.530 and —0.535° C. Dahlberg, Adams, and Held (7) in 1953 reported an average of —0.540° C. for the freezing points of market milk from eight large cities throughout the United States. Of the 135 values only 13.3 per cent were lower than —0.550° C., whereas 27.5 per cent were higher than —0.535° C. Except for the sample from one city, the average freezing point was statistically higher than —0.550° C.

There is also a question as to the normal range of freezing points. The official method allows a tolerance of 3 per cent added water which is equivalent to fixing —0.5335° C. as the upper freezing-point limit. A number of investigators have reported freezing points above this value for samples of known purity.

Buchanan and Lowman (5, 6) in 1926 and 1929 reported seasonal variations in the freezing point of milk and suggested that changes in feed and/or meteorological conditions might be contributing factors. Elsdon and Walker (8) gave a review of variations observed by several investigators prior to 1935. Koenig (12) in 1941 reported a range of freezing points from —0.500 to —0.529° C. for cows on a poor quality ration. However, when they were fed fresh cut grass they gave a range from —0.581 to —0.591° C. for the first few days. Within a week they returned to normal.

Aschaffenburg and Rowland (1), investigating the freezing point of milk from a 30-cow herd, reported values of —0.525, —0.528, —0.526, and —0.531° C.

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for evening milk and —0.555, —0.556, —0.560, and 0.566° C. for the corresponding morning milk collected on four different dates. During this period the herd was housed from about 4 P.M. to 9 A.M. without access to water. Then the animals were turned out to pasture where they had free access to water. Under these conditions freezing points of —0.553 and —0.542° C. were observed for evening milk and —0.546 and —0.543° C. for morning milk.

Hillman, Provan, and Steane (10) also observed considerable difference in freezing points between morning and evening milk. Combined evening milk from five cows gave a range from —0.515 to —0.527° C. when tested over an 8-week period. The corresponding range for morning milk was from —0.560 to —0.550° C.

In 1952 Lythgoe (15) presented a summary of the freezing points of 354 samples collected over a period of years by the Food and Drug Division of the Massachusetts Department of Public Health. His data show a range of freezing points from —0.510 to —0.585° C.

Aschaffenburg and co-workers (2, 3) in England have made a very extensive study of freezing-point variations. In a study of 1,087 morning and 1,051 evening samples they found a range of freezing points from —0.527 to —0.575° C. They found seasonal trends, both in average values and in the differences between morning and evening milk. They also observed a small, but significant, difference between the milk of Shorthorn and Guernsey cows. They state that their results indicate that the United States standard, which fixes the upper limit for unwatered milk at —0.5335° C., is too stringent. They suggest that the English practice of fixing the upper limit at —0.530° C. is a more reasonable value.

These published data indicate a need for an extensive study to definitely establish a valid average and normal range for the freezing point of the present milk supply in our country. A more complete understanding of the factors affecting the freezing point of cow's milk is needed to determine the significance of any particular set of freezing-point values. This study was undertaken to determine the effect of some of the kinds of changes in dairy cattle management which have occurred in the last 30 years.

EXPERIMENTAL

The modified cryoscopic procedure described in the preceding paper (17) was followed in making all of the freezing-point determinations. A number of the samples were tested also by the Hortvet procedure and all the results agreed within 0.002° C.

Two series of experiments were conducted: one designed to compare the freezing points of different breeds and the other to compare the freezing points for a group of cows on different rations. Five Holstein and five Brown Swiss were chosen from the University herd for the breed comparison. These cows were housed in the same barn in adjacent stalls. Milking was begun at 5:30 A.M. and 4:30 P.M. Samples were collected weekly during January, February, and March. The samples were taken directly from the barn to the laboratory and

held at 35 to 38° F. until tested. The samples were tested within 18 hours. At the beginning of the experiment all samples were tested within 6 hours after milking, but since it was found that under this method of handling the freezing point did not decrease in the first 24 hours, it was considered unnecessary to test the samples immediately.

During the first 4 weeks of this experiment, the cows were fed a good quality hay, corn silage, and a 16 per cent dairy milk ration. During the latter part of the experiment, part of the corn silage was replaced with grass silage. Originally the corn silage was fed at about 9 A.M. and left in the manger until about 3 P.M., at which time any remainder was removed and hay was put in. In the latter phase of the experiment grass silage was fed at 9 A.M. and corn was given at 3 P.M. Hay was given after the evening milking.

The second experiment was set up to study the effect of three different rations on freezing points. For this experiment five Holstein cows were chosen from the University herd. In the first phase of the experiment the cows were fed only mixed hay. They were kept on this ration for several weeks until their food intake and production had become stabilized. After the initial adjustment period the ration schedule was set up as follows:

First hay period—1st and 2nd wk. (March 28-April 9)
First hay-grain period—3rd and 4th wk. (April 10-April 23)

Second hay period—5th and 6th wk. (April 24-May 7)
Second hay-grain period—7th and 8th wk. (May 8-May 21)

Third hay period—9th and 10th wk. (May 22-June 4)
Pasture period—11th through 14th wk. (June 5-June 30)

After the first 2 weeks, part of the hay was replaced by ground corn meal, at the rate of 1 lb. per 3.2 lb. of fat corrected milk with an "equivalent amount" of hay being removed. The "equivalent amount" of hay for cows 142, 158, and 168 was computed on a net energy basis, whereas it was computed on a total digestible nutrients (TDN) basis for cows 93 and 156. For the second hay-grain period the hay equivalence was computed from TDN values for cows 142, 158, and 168, and from net energy values for the other two. For this experiment, water meters were placed on each cow's drinking cup, and readings were taken before each milking. The cows did not have access to any other water supply. While on pasture all cows were fed silage and a 16 per cent dairy milk ration.

The ration changes were always made on Wednesday afternoons. Samples of milk were collected mornings and evenings on Tuesday, Wednesday, Thursday, and Friday of each week. The samples were taken on these days so as to determine whether the change of rations had an immediate effect on the freezing points. The values reported for the hay rations represent samples collected during the 2 weeks of the first hay period and the 2 weeks of the second hay period. The values reported for the hay-grain ration represent samples collected during the first 2-week period on this ration. In addition, composite samples were collected morning and evening during the third hay period and

second hay-grain period. The values reported for the pasture period represent samples collected during the first, second, and fourth week of this period.

RESULTS AND DISCUSSION

The results of the comparison of the freezing points of the milk from Brown Swiss and Holstein cows are shown in Table 1. Originally, there were five cows

TABLE 1

Comparison of the effect of breed and ration on the freezing-point depression of cow's milk

Breed	Cow No.		Ration No. 1					Ration No. 2				
			1-22	1-29	2-5	2-12	Av.	2-19	2-27	3-6	3-28	Av.
			(° C.)	(° C.)	(° C.)	(° C.)	(° C.)	(° C.)	(° C.)	(° C.)	(° C.)	(° C.)
Brown Swiss	1	AM	0.513	0.529	0.541	0.530	0.528	0.527	0.529	0.544	0.543	0.536
		PM	0.529	0.531	0.531	0.521	0.528	0.528	0.535	0.523	0.542	0.532
	2	AM	0.539	0.535	0.544	0.531	0.537	0.529	0.543	.....	.....	0.536
		PM	0.529	0.529	0.532	0.525	0.529	0.535	0.536	.....	.....	0.536
	3	AM	0.544	0.530	0.543	0.528	0.536	0.539	0.535	0.537	0.544	0.539
		PM	0.530	0.531	0.543	0.534	0.534	0.546	0.533	0.529	0.544	0.538
	4	AM	0.530	0.549	0.529	0.526	0.533	0.536	0.530	0.535	0.534	0.534
		PM	0.525	0.529	0.527	0.529	0.528	0.536	0.537	0.535	0.546	0.538
	5	AM	0.545	0.551	0.548	0.542	0.546	0.542	0.536	0.543	0.535	0.539
		PM	0.545	0.543	0.541	0.540	0.541	0.531	0.555	0.543	0.543	0.540
						Av. AM	0.536					0.537
						Av. PM	0.532					0.537
						Av. Daily	0.534					0.537
Holstein	7	AM	0.532	0.545	0.557	0.541	0.544	0.536	0.546	0.537	0.534	0.538
		PM	0.542	0.531	0.541	0.536	0.538	0.529	0.544	0.539	0.545	0.539
	8	AM	0.555	0.545	0.550	0.527	0.544	0.535	0.543	.....	.....	0.539
		PM	0.523	0.551	0.530	0.520	0.531	0.555	0.543	.....	.....	0.549
	12	AM	0.531	0.531	0.535	0.534	0.535	0.537	0.539	0.535	0.537	0.537
		PM	0.530	0.544	0.535	0.533	0.535	0.536	0.553	0.557	0.553	0.550
	13	AM	0.550	0.550	0.555	0.548	0.552	0.539	0.540	0.543	0.545	0.542
		PM	0.542	0.544	0.539	0.534	0.540	0.547	0.563	0.545	0.555	0.552
						Av. AM	0.544					0.539
						Av. PM	0.536					0.548
						Av. Daily	0.540					0.543
						Grand Av. AM	0.540					0.538
						Grand Av. PM	0.534					0.542
						Grand Av. Daily	0.537					0.540

of each breed, but one of the Holsteins became sick and had to be dropped from the experiment. Although the rations were changed without advance notice to the authors, it is felt that this change was fortunate since it revealed unexpected results. A statistical analysis of variance of these data revealed a significant difference at the 1 per cent level between breeds, rations, and cows.

The average freezing point of morning milk was lower than that of evening milk on ration 1, whereas on ration 2 the reverse was true. It is also worth noting

that the morning-evening differences are not the same for both breeds. These two observations may partially explain the contradictory reports in the literature regarding the freezing point of morning versus evening milk.

Examination of the data in Table 1 reveals that only two samples of Brown Swiss milk and nine samples of Holstein milk had freezing points below —0.550° C. This represents 2.6 and 15.0 per cent, respectively, of the samples of each breed. Of the total of 136 samples only 11, or 8.1 per cent, had freezing points below —0.550° C. Only once (cow No. 13, on February 27) did the daily average freezing point of milk from an individual cow fall below —0.550° C. Forty-three samples, or 31.6 per cent, had freezing points above —0.5335° C.

The fact that breed differences were exhibited even under identical environment emphasizes a need for further study to determine the average freezing point of our present milk supply. This is particularly true in this country because of the changes in the proportions of different breeds which have taken place since Hortvet published his data.

In the second phase of this research a more extensive study was made of the effect of rations on freezing points. For this study, five Holstein cows were used. The effect of ration on the differences between the freezing points of morning and evening milk is shown in Table 2. The freezing points of the

TABLE 2

Effect of ration changes on the average freezing points of morning and evening milk [a]

Cow No.	Hay-grain		Hay		Pasture	
	AM	PM	AM	PM	AM	PM
	(° C.)	(° C.)	(° C.)	(° C.)	(° C.)	(° C.)
93	—0.533	—0.539	—0.536	—0.549	—0.547	—0.544
142	—0.527	—0.535	—0.532	—0.542	—0.542	—0.537
156	—0.535	—0.537	—0.543	—0.546	—0.550	—0.541
158	—0.532	—0.538	—0.535	—0.544	—0.549	—0.541
168	—0.533	—0.535	—0.538	—0.545	—0.549	—0.547
Av.	—0.532	—0.537	—0.537	—0.545	—0.547	—0.542
High [b]	—0.524	—0.532	—0.527	—0.535	—0.535	—0.530
Low [b]	—0.534	—0.544	—0.553	—0.563	—0.565	—0.563

[a] The individual values for the hay-grain and pasture rations represent the average freezing point of 7 samples.
The values for the hay period represent the average of 9 samples.
[b] These values represent the high and low values for the individual samples.

samples taken the first day after each ration change are not included in the results. In general, the morning milk on the day following the ration change had "abnormal" freezing points, compared with the rest of the period. The corresponding evening samples were more nearly "normal," but they also were omitted from the calculation of averages. The second day after the ration changes the freezing points were "normal," compared to the rest of the period.

The individual values in Table 2 for the hay-grain and pasture rations represent the average freezing point of seven samples taken throughout these periods. The values for the hay rations represent nine samples, four collected

during the first hay period and five during the second hay period. Composite samples from the five cows were collected both morning and evening during the third hay period and the second hay-grain period. The average values for the three hay periods were —0.538, —0.535, and —0.535° C. for morning milk and —0.546, —0.546, and —0.548° C. for the corresponding evening milk. The average values for the two hay-grain periods were —0.532 and —0.531° C. for morning milk and —0.537 and —0.538° C. for the corresponding evening milk. The fact that the freezing points were essentially the same for each period on a given ration indicates that the observed freezing points were characteristic for the rations.

The results in Table 2 provide additional evidence of the differences between the freezing points of morning and evening milk. With the hay and hay-grain rations the freezing point was highest in the morning, whereas with pasture feeding it was highest in the evening. It is also interesting to note that the freezing-point range is least on the hay-grain ration and greatest on the pasture.

There was no apparent difference between the freezing points of the milk of cows for which the equivalence of hay and grain was computed on a net energy basis and those for which the equivalence was computed on a TDN basis.

A comparison of freezing points and weight of milk and water intake is shown in Table 3. The values for the hay-grain ration represent the averages

TABLE 3

The effect of type of ration on water intake, milk production, and freezing point of milk

Cow No.	Hay ration			Hay-grain ration		
	Water intake	Wt. of milk	Freezing point	Water intake	Wt. of milk	Freezing point
	(lb.)	*(lb.)*	*(° C.)*	*(lb.)*	*(lb.)*	*(° C.)*
156	169.3	32.82	—0.545	130.1	39.56	—0.537
93	161.0	35.61	—0.543	106.7	39.25	—0.536
142	152.6	36.97	—0.537	87.6	36.96	—0.532
158	126.7	26.84	—0.540	80.9	28.99	—0.536
168	121.7	16.70	—0.541	85.1	17.59	—0.535
Av.	149.3	29.79	—0.541	100.9	32.27	—0.535

for the second week of the first hay-grain period. The corresponding values for the hay ration are averages of the second week of both the first and second hay periods. In other words, the values for the hay ration represent averages for the second and sixth week of the experiment, whereas the values for the hay-grain represent averages for the fourth week of the experiment. Therefore, any changes due to stage of lactation should be balanced out.

The results in Table 3 show that the water intake is considerably less on the hay-grain ration than on the hay ration. The decrease in water intake when the cows were changed from hay to the hay-grain ration was closely associated with the per cent decrease in hay intake. This reduction in water intake is shown to be associated with a rise in the freezing point and amount of milk. This observation is somewhat surprising. However, since these cows had free

access to water, the increase in water intake on the hay ration may merely suggest a greater physiological requirement for water. It might be assumed that when the requirement is greatest the cow is least capable of drinking adequate amounts of water, hence milk production is reduced with a concurrent reduction in freezing point. In this sense variations in water intake would appear to be an effect of ration changes rather than a cause of freezing point and/or production changes. However, if access to water is limited, water intake may become a critical factor. It would seem reasonable to expect that restriction of water intake would be more important when the physiological requirement is greatest. In the days when Hortvet made his study, hay probably constituted a greater portion of the dairy ration and water was probably less accessible than now. Therefore, the freezing points may have been lower then.

The differences in the freezing-point distributions for the three different rations are shown in Figure 1. The data represent the individual daily averages which were computed from the individual values obtained from morning and evening samples. The daily averages were used rather than values for individual milkings since these are more comparable to milk-plant samples. The data for the hay-grain and pasture rations represent seven daily values for each of the

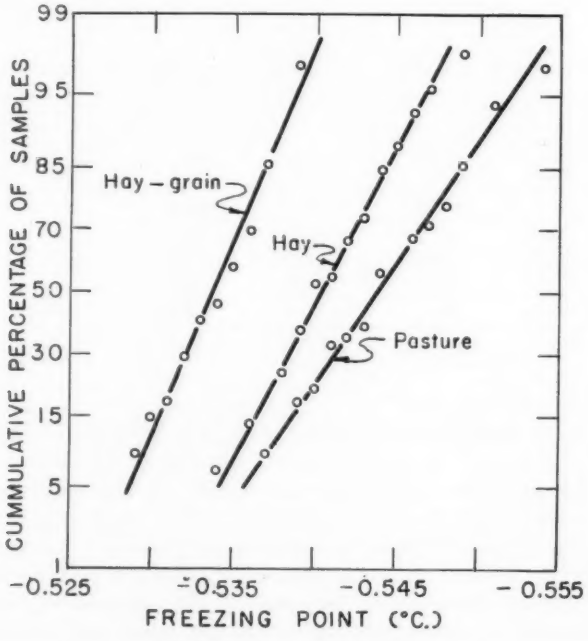

FIG. 1. A comparison of the effect of rations on the frequency distributions of the freezing points of milk expressed as the cumulative percentages of the samples with freezing points at or above the temperatures shown on the graph.

five cows, and the data for the hay ration represent nine daily values for each cow. The results are shown on an arithmetic probability scale in order to show clearly the differences in the distribution patterns.

These patterns vary in two ways: first, the values are distributed about different averages; secondly, the range of distribution is different. The results show that 90 per cent (i.e., from 5 to 95 per cent) of the values obtained on the hay-grain rations range from —0.529 to —0.539° C.; on hay the corresponding range was from —0.534 to —0.547° C.; and on pasture from —0.536 to —0.552° C. Although there are not sufficient data to warrant definite conclusions, the fact that the freezing point is least variable on the hay-grain ration might be attributable to the relative uniformity of this ration. On the other hand, the relatively wide range on the pasture ration might be partially attributable to the variations in the condition of the pasture. This portion of the experiment was conducted throughout the month of June, which was quite dry. At the beginning, the pasture was lush, but by the end of the month it had noticeably dried up. Some of the variability during the pasture period, however, may be due to variations in temperature and humidity.

The fact that the replacement of part of the hay with grain raises the freezing point is of particular interest because in the 30 years since Hortvet published his data the per cent of grain in the dairy ration has been increased considerably. Therefore, it would not be surprising if the average freezing point has shifted upward. However, a more extensive study of a variety of rations is necessary before any general conclusions can be drawn.

As may be seen in Figure 1, approximately 45 per cent of the daily averages for the hay-grain ration was above the "official upper limit" of —0.5335° C., whereas less than 5 per cent of the samples on the hay and pasture rations was above this value. It is also worth noting that none of the values on the hay-grain ration were below —0.550° C. Less than 1 per cent of the values for the hay ration and less than 10 per cent of the values for the pasture ration were below —0.550° C.

A statistical analysis of the freezing points obtained on the three rations shows that the difference between the hay-grain ration and either the pasture or hay ration is significant at the 1 per cent level. The difference between the freezing points obtained on hay and on pasture is significant at the 5 per cent level. The difference between the morning and evening milk for a given ration also was significant at the 5 per cent level. However, a statistical analysis of the freezing points of all the morning and evening samples did not reveal a significant difference. This may be attributed to the fact that the morning samples had higher freezing points than the evening samples for the hay and hay-grain rations, but the reverse was true for the pasture ration, as was shown in Table 2.

Between December 1951 and August 1952, the freezing points of 594 authentic individual cow samples were determined. These samples represented milk from 25 cows of the University herd and 11 cows of another herd. The freezing points ranged from —0.513 to —0.565° C. with an average of —0.539°

C. The freezing points of 27 per cent of the samples were above —0.5335° C., but only 9 per cent were below —0.550° C. The collaborative study of Bailey (4) in 1922 revealed a range of —0.523 to —0.580° C. with an average of —0.545° C. The fact that the differences between the extremes are of the same order of magnitude supports the assumption that the freezing points of milk vary within relatively narrow limits. For this reason, the use of cryoscopic procedure for the detection of adulteration is considered a basically sound practice. However, the differences in the averages indicate a need for a more extensive study to establish the correct range of values for the present milk supply in this country. Furthermore, it should be pointed out that the assumption that —0.550° C. is the correct average freezing point is based on data published before the development of the official method. This assumption is not confirmed by the data found in the literature on freezing points as determined by the official method.

SUMMARY

Variations in the freezing points of cow's milk were observed under a variety of conditions. Statistically significant differences were observed between Holstein and Brown Swiss cows and between rations. The freezing points of morning and evening samples show significant differences. Under certain conditions the morning samples had higher freezing points than evening samples, whereas under other conditions the evening samples had higher freezing points. The average freezing point of 594 samples of authentic individual cow samples was found to be —0.539° C. with a range from —0.513 to —0.565° C. The range of freezing points varied with the ration.

The implications of the results of this study are discussed in detail.

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REFERENCES

(1) Aschaffenburg, R., and Rowland, S. J. Abnormal Freezing-Point of Milk. Chemistry and Industry, pp. 636-637. 1950.

(2) Aschaffenburg, R., and Temple, P. L. The Freezing-Point of Milk. I. The Freezing-Point and Solids-Not-Fat Content of the Milk of Individual Cows Throughout a Period of Lactation. J. Dairy Research, 12: 315-321. 1941.

(3) Aschaffenburg, R., and Veinoglou, B. C. The Freezing-Point of Milk. II. The Influence of Various Factors and their Bearing on the Detection of Added Water. J. Dairy Research, 13: 267-280. 1944.

(4) Bailey, E. M. Cryoscopy of Milk. J. Assoc. Offic. Agr. Chemists, 5: 484-497. 1922.

(5) Buchanan, J. H., and Lowman, O. E. Some Observations on the Freezing-Point of Milk. J. Dairy Sci., 9: 192-202. 1926.

(6) Buchanan, J. H., and Lowman, O. E. Seasonal Variations in the Freezing-Point of Milk. J. Dairy Sci., 12: 484-490. 1929.

(7) DAHLBERG, A. C., ADAMS, H. S., AND HELD, M. E. Sanitary Milk Control and its Relationship to the Sanitary, Nutritive and Other Qualities of Milk. National Research Council Publication. No. 250. 1953.

(8) ELSDON, G. D., AND WALKER, G. H. *Richmond's Dairy Chemistry*, 4th ed. J. P. Lippincott Co., Philadelphia, London, and Montreal. 1942.

(9) HENDERSON, J. B., AND MESTON, L. A. The Freezing-Point of Milk. Chem. News, **110**: 283-284. 1914.

(10) HILLMAN, H. C., PROVAN, A. L., AND STEANE, E. Abnormal Freezing-Point of Milk. Chemistry and Industry, pp. 333-334. 1950.

(11) HORTVET, J. The Cryoscopy of Milk. J. Ind. Eng. Chem., **13**: 198-208. 1921.

(12) KOENIG, W. Regelwidrige Gefrierpunkte unverfälschter Milch. Z. Untersuch. Lebensmitt., **81**: 481-488. 1941.

(13) KRIENKE, W. A., AND ARRINGTON, L. R. A Re-evaluation of the Hortvet Formula and Freezing-Point Value of Milk in Estimating the Percentage of Added Water. J. Dairy Sci., **32**: 699. 1949.

(14) LAMPERT, L. M. The Freezing Point of Milk. J. Assoc. Offic. Agr. Chemists, **22**: 768-771. 1939.

(15) LYTHGOE, H. C. Cryoscopic Determination of Added Water in Milk. J. Assoc. Offic. Agr. Chemists, **35**: 442-453. 1952.

(16) PALEY, C., AND TZALL, B. An Inquiry into the Interpretation of the Freezing Points of Milk Obtained by the Hortvet Cryoscope and its Relationship to Present-Day Market Fluid Milk. 24th Ann. Rpt. of N. Y. State Assoc. of Milk Sanitarians. 1950.

(17) SHIPE, W. F., DAHLBERG, A. C., AND HERRINGTON, B. L. A Semi-Automatic Cryoscope for Determining the Freezing Point of Milk. J. Dairy Sci., **36**: 916-923. 1953.

(18) STUBBS, J. R., AND ELSDON, G. D. The Examination of 1000 Milk Samples by the Hortvet Freezing Point Process. Analyst, **34**: 146-152. 1934.

TABLE 1

Seasonal mean [a] values for various physiological and semen characteristics of dairy bulls

Breed	No. of bulls	Season	Respiration rate per min.	Heart rate per min.	Body temperatures	Abnormal spermatozoa	Motile spermatozoa	Concentration spermatozoa
					(° F.)	*(%)*	*(%)*	*(millions / ml.)*
Jersey	4	Winter	15(0.60)	59(3.73)	100.9(0.04)	15(0.66)	65(0.79)	1501(59.58)
		Spring	22(1.14)	64(2.07)	101.0(0.32)	14(0.75)	61(0.65)	1350(57.35)
		Summer	55(2.47)	61(1.16)	101.1(0.08)	16(0.98)	58(1.38)	1477(68.81)
		Fall	28(1.67)	63(0.51)	101.1(0.06)	17(0.83)	56(2.21)	1285(86.40)
		Year	31(1.36)	63(0.87)	101.0(0.04)	15(0.42)	60(0.68)	1418(33.74)
Guernsey	5	Winter	14(3.60)	50(1.44)	100.8(0.04)	18(1.15)	59(0.93)	1038(55.70)
		Spring	21(0.62)	58(2.53)	101.1(0.40)	14(0.61)	62(0.75)	1019(44.32)
		Summer	55(1.87)	56(0.71)	101.1(0.11)	18(0.83)	56(0.69)	1146(37.89)
		Fall	36(0.65)	54(0.89)	101.0(0.49)	19(0.95)	53(1.04)	962(57.82) [b]
		Year	33(1.26)	56(0.88)	101.0(0.03)	17(0.46)	58(0.47)	1047(24.46)
Holstein	5	Winter	14(0.35)	48(1.27)	100.9(0.05)	16(0.88)	61(0.55)	1136(49.43)
		Spring	24(1.03)	63(1.26)	101.1(0.04)	14(0.47)	60(0.65)	912(34.84)
		Summer	55(0.91)	55(0.76)	101.3(0.09)	23(1.37)	50(1.02)	1003(43.04)
		Fall	31(1.73)	56(0.86)	100.9(0.06)	21(1.64)	48(1.69)	931(62.94) [b]
		Year	32(1.34)	57(0.60)	101.1(0.02)	19(0.62)	55(0.62)	999(24.51)
All bulls	14	Winter	14(0.81)	52(0.45)	100.9(0.06)	17(0.54)	62(0.48)	1215(34.56)
		Spring	22(1.80)	62(1.15)	101.1(0.02)	14(0.34)	61(0.41)	1070(29.07)
		Summer	55(1.41)	57(0.53)	101.2(0.05)	19(0.67)	54(0.41)	1190(32.01)
		Fall	32(1.09)	58(0.71)	101.0(0.04)	19(0.90)	52(0.98)	1030(40.77)
		Year	32(0.77)	58(0.47)	101.0(0.02)	17(0.31)	57(0.33)	1133(9.50)

[a] Standard errors of the means are indicated in parenthesis.

[b] One bull of each of the Guernsey and Holstein breeds showed marked decreases in concentration of spermatozoa during the late summer and fall periods.

TABLE 2

Seasonal summary of fertility and climatological data

Seasons		Breed				Climatic observations		
		Jersey	Holstein	Guernsey	All Breeds	Mean max. temp.	Mean min. temp.	Mean vapor pressure
						(° F.)	*(° F.)*	*(mm. Hg.)*
Winter	No. ejaculates	52	63	55	170	60.0	42.7	8.8
	% ejaculates shipped	75.0	90.5	89.1	85.3			
	Services	1,567	1,654	1,758	4,979			
	% Non returns	66.4	74.8	69.3	70.2			
Spring	No. ejaculates	45	60	60	165	74.8	56.4	13.4
	% ejaculates shipped	77.8	91.7	91.7	87.9			
	Services	1,256	997	1,605	3,858			
	% Non returns	69.1	70.9	68.5	69.3			
Summer	No. ejaculates	52	65	60	177	90.2	73.0	22.0
	% ejaculates shipped	73.1	72.3	96.7	80.8			
	Services	1,441	725	1,876	4,042			
	% Non returns	67.6	65.1	64.8	65.9			
Fall	No. ejaculates	33	55	44	132	75.9	55.9	14.6
	% ejaculates shipped	57.6	54.5	86.4	65.9			
	Services	1,064	1,065	2,244	4,373			
	% Non returns	67.3	66.0	63.6	65.1			
Year	No. ejaculates	182	243	219	644	70.4	57.5	13.9
	% ejaculates shipped	72.0	77.8	91.3	80.7			
	Services	5,328	4,441	7,483	11,679			
	% Non returns	67.5	70.2	66.3	67.7			

among breeds. The year during which this study was conducted was an extreme one for the area; the temperature ranged from a minimum of 13° F. on February 2 and 3 to a maximum of 102° F. on August 13. The response of individual bulls to summer conditions was very variable, there being considerable overlapping among the breeds with respect to ability to maintain body temperature and fertility. This within-breed variation probably explains the lack of significance in breed differences. It would appear that under the climatic conditions of this study the ability of bulls to resist high temperatures and humidities is more an individual than a breed characteristic.

The data on pulse rate, body temperature, respiration rate, maximum temperature, mean temperature, and vapor pressure also were summarized according to maximum temperature intervals. Analysis of variance of the data on this basis showed (a) no significant breed differences in body temperature, (b) significant breed differences in respiration rate only at the 80-85° F. temperature range, and (c) significant (40-60, 60-70, and 70-80° F.) or highly significant (80-85, 85-90, and 90-95° F.) breed differences in pulse rate in all temperature ranges.

Coefficients of correlation calculated between each of the physiological and climatological observations are shown in Tables 3 and 5. The zero order correla-

TABLE 3

Zero order coefficients of correlation between climatic measurements and pulse rate and body temperature calculated at various maximum temperature intervals

Max. temp. interval	Physiological measurements	Climatic measurements		
		Av. weekly max. temp.	Av. weekly mean temp.	Av. weekly vapor pressure
(° F.)				
40-60	Pulse rate	− 0.26	− 0.26	− 0.26
	Body temp.	0.38[b]	0.32[b]	0.27[b]
60-70	Pulse rate	− 0.09	0.15	− 0.13
	Body temp.	0.05	0.08	− 0.04
70-80	Pulse rate	0.08	0.08	0.17
	Body temp.	0.23[a]	0.10	0.04
80-85	Pulse rate	0.08	− 0.04	− 0.22[b]
	Body temp.	0.00	0.05	0.05
85-90	Pulse rate	− 0.05	− 0.26[b]	0.20[a]
	Body temp.	0.10	0.03	0.32[b]
90-95	Pulse rate	0.17	0.14	0.48[b]
	Body temp.	0.18	0.14	0.30[b]

[a] Significant at 5% level.
[b] Significant at 1% level.

tions involving respiration rate were the only ones which were consistently statistically significant at the various temperature intervals. To further define these relationships, first and second order partial correlations and multiple correlations were calculated and are shown in Table 4. From the second order partials, it would appear that mean temperature and vapor pressure are more closely related to respiration rate over the entire temperature range than is maximum temperature. The apparent inconsistencies which appear, particularly in the vapor pressure correlations within the 70-80° F. range, are difficult to ex-

TABLE 4

Coefficients of correlation between climatic measurements and respiration rate (Y) calculated at various maximum temperature intervals

	Independent of	Maximum Temperature Interval (° F.)					
		40-60	60-70	70-80	80-85	85-90	90-95
Maximum temp. (X_1)	.....	0.47[b]	0.34[b]	0.31[b]	0.08	0.12	0.40[b]
	X_2	−0.23	0.12	−0.27[a]	−0.01	−0.01	0.25[a]
	X_3	0.15	0.14	0.20	0.08	0.11	0.44[b]
	X_2, X_3	−0.32[a]	−0.02	0.07	−0.01	0.08	0.35[b]
Mean temp. (X_2)	.....	0.52[b]	0.40[b]	0.52[b]	0.24[b]	0.26[b]	0.32[b]
	X_1	0.34[b]	0.25[b]	0.50[b]	0.22[a]	0.23	−0.10
	X_3	0.27[a]	0.23[b]	0.67[b]	−0.47[b]	0.08	0.34[b]
	X_1, X_3	0.60[b]	0.21	0.92[b]	−0.51[b]	0.03	−0.19
Vapor pressure (X_3)	.....	0.46[b]	0.43[b]	0.27[b]	0.52[b]	0.40[b]	0.43[b]
	X_1	0.12	0.29[b]	0.09	0.52[b]	0.40[b]	0.48[b]
	X_2	−0.06	0.28[b]	−0.55[b]	0.63[b]	0.33[b]	0.44[b]
	X_1, X_2	−0.23	0.25[b]	−0.25[b]	0.65[b]	0.33	0.50[b]
Multiple correlations		$RY \cdot X_1X_2 =$ 0.56[b]	$RY \cdot X_2X_3 =$ 0.48[b]	$RY \cdot X_2X_3 =$ 0.70[b]	$RY \cdot X_2X_3 =$ 0.66[b]	.....	$RY \cdot X_1X_3 =$ 0.59[b]

[a] Significant at 5% level.
[b] Significant at 1% level.

plain at the present time. However, because of the small number of animals involved, these results can be considered only as indicative. Nevertheless, it would appear that humidity is more important in heat tolerance reactions than other authors (6, 13, 14) have indicated. The fact that most of the earlier workers have based their observations on relative instead of absolute humidity may explain the difference between their results and those obtained in this study.

Figure 1 shows 6-week moving averages of climatic measurements and fer-

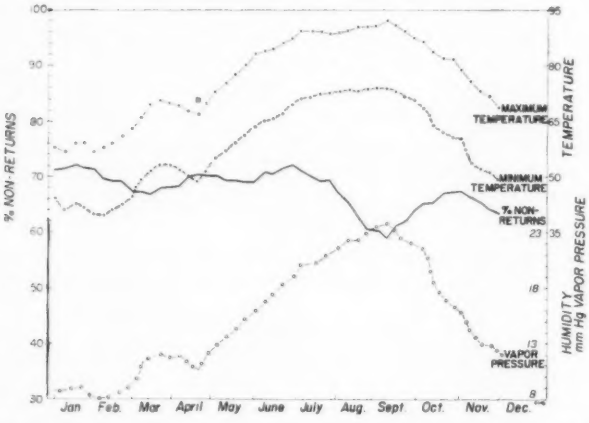

FIG. 1. Six-week moving averages of climatic observations and fertility data plotted as last day of each six-week period.

tility. The 6-week period was selected since it is the approximate interval from spermatozoa formation to ejaculation. Therefore, semen produced during any 1-week period would tend to show the combined effects of climatic conditions experienced during the past 6 weeks. It may be noted that, on this basis, fertility was generally high during the winter, spring, and early summer seasons. It began to drop during late July and early August and in early September showed a low, which was followed by a rise and subsequent decline in November. Coefficients of correlation calculated from these data, all significant at the 1 per cent level, were as follows: maximum temperature and fertility, –0.46; minimum temperature and fertility, –0.45; vapor pressure and fertility, –0.55; maximum temperature and vapor pressure, 0.98, and minimum temperature and vapor pressure, 0.99. The extremely high correlations among the climatic measurements on the basis of 6-week moving averages explain the similarity of the fertility correlations. However, here again the climatic condition showing the highest degree of relationship to fertility is vapor pressure (absolute humidity).

When the data utilized in the calculations were limited to the period during which the 6-week moving average maximum temperature was 80° F. or above, the correlation between maximum temperature and fertility was increased to only –0.53.

It may be noted that the fertility data reported in this study are based on selected semen samples and therefore do not represent the total fertility of the bulls for the period of study. The use of minimum quality standards for the selection of semen to be used for artificial insemination eliminates many of the low fertility ejaculates and tends to limit the seasonal climatic effect upon the estimated fertility of bulls in such service. On this basis the relationship between climatic factors and fertility shown in this study probably is an underestimation of the true climatic effect on natural fertility. This thesis is supported by the seasonal per cent of ejaculates shipped, which was lowest for all breeds during the fall period, indicating the possibility of a carry-over effect on semen quality.

SUMMARY AND CONCLUSIONS

The physiological reactions, semen characteristics, and fertility of 14 dairy bulls were studied in conjunction with climatic conditions for 53 weeks in a subtropical environment. Under the conditions of this study it was not possible to show statistically significant breed differences in respiration rate, body temperature, or fertility of bulls of the Holstein, Guernsey, and Jersey breeds. When the data were grouped according to maximum temperature intervals, only respiration rate was found to be consistently related to climatic conditions. Absolute humidity and mean environmental temperature were found to be more highly correlated with respiration rate over the entire temperature range than was maximum temperature. Six-week moving averages of climatic measurements and fertility in artificial breeding use were calculated. On this basis the following coefficients of correlation between fertility and climatic measurements were obtained: maximum temperature –0.46; minimum temperature –0.45; vapor pressure –0.55. These correlations are significant at the 1 per cent level.

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REFERENCES

(1) Anderson, J. The Semen of Animals and its Use for Artificial Insemination. Imp. Bur. An. Breeding and Genetics, T. C. 1945.

(2) Branton, C., D'Arensbourg, G., and Johnston, J. E. Semen Production, Fructose Content of Semen and Fertility of Dairy Bulls as Related to Sexual Excitement. J. Dairy Sci., **35**: 801-807. 1952.

(3) Branton, C., James, C. B., Patrick, T. E., and Newsom, M. H. The Relationship between Certain Semen Quality Tests and Fertility and the Interrelationship of these Tests. J. Dairy Sci., **34**: 310-316. 1951.

(4) Dordick, I. L. The Effect of High Temperature and Humidity upon Cattle. Acta Tropica, **6**: 221-245. 1949.

(5) Erb, R. E., Wilbur, J. W., and Hilton, J. H. Some Factors Affecting Breeding Efficiency in Dairy Cattle. (Abs.) J. Dairy Sci., **23**: 549. 1950.

(6) Gaalaas, R. F. Effect of Atmospheric Temperature on Body Temperature and Respiration Rate of Jersey Cattle. J. Dairy Sci., **28**: 555-563. 1945.

(7) JONES, J. R., DOUGHERTY, R. W., AND HAAG, J. R. Reproductive Performances in Dairy Cattle. Ore. State College Bull. 395. 1941.

(8) MERCIER, E., AND SALISBURY, G. W. Seasonal Variations in Hours of Daylight Associated with Fertility Level of Cattle Under Natural Breeding Conditions. J. Dairy Sci., 30: 749-756. 1947.

(9) MERCIER, E., AND SALISBURY, G. W. Fertility Levels in Artificial Breeding Associated with Season, Hours of Daylight, and Age of the Cow. J. Dairy Sci., 30: 817-825. 1947.

(10) MORGAN, R. F., AND DAVIS, H. P. Influence of the Age of the Cow and Season of the Year on the Sex Ratio of Calves and Service per Conception. Neb. Agr. Expt. Sta. Research Bull. 104. 1938.

(11) SHAFFER, H. E., AND ALMQUIST, J. O. Relation of the Eosin-Aniline Blue Staining Method to the Quality of Bull Semen. (Abs.) J. Dairy Sci., 32: 723. 1949.

(12) SEATH, D. M. Heritability of Heat Tolerance in Dairy Cattle. J. Dairy Sci., 30: 137-144. 1947.

(13) SEATH, D. M., AND MILLER, G. D. The Relative Importance of High Temperature and High Humidity as Factors Influencing Respiration Rate, Body Temperature and Pulse Rate of Dairy Cows. J. Dairy Sci., 29: 465-472. 1946.

(14) SEATH, D. M., AND MILLER, G. D. Heat Tolerance Comparison Between Jersey and Holstein Cows. J. Animal Sci., 6: 24-34. 1947.

(15) SNEDECOR, G. W. *Statistical Methods.* 4th ed. The Iowa State College Press, Ames. 1946.

(16) WEETH, H. J., AND HERMAN, H. A. The Relationship between Semen Quality and Conception Rate in Artificial Insemination of Dairy Cattle. Mo. Agr. Expt. Sta. Research Bull. 447. 1949.

THE PRESENCE OF HIPPURIC ACID IN MILK[1]

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During research on the ether-extractable acidic material from heated milk, an unidentified crystalline substance was recovered. Its physical and chemical properties suggested that it was hippuric acid (benzoyl glycine). Before investigating the matter further, a fairly comprehensive search of the literature was made concerning the possible presence of this compound in milk. One such reference was found. While concentrating the biotin-containing fraction of milk, Karabinos and Dittiner (5) obtained 11 g. of hippuric acid from 1,160 kg. of milk, equivalent to a concentration of approximately 1 mg./100 ml. However, the authors state that the concentration may have been considerably greater since it is not known how much of the compound was lost in the process of isolation. In order to supplement this limited information, studies were undertaken to confirm the presence and determine the concentration of hippuric acid in milk.

EXPERIMENTAL

Identification of hippuric acid in milk. Raw skimmilk was forewarmed to 60° C. and condensed 3 to 1 in a stainless steel vacuum pan at a temperature of 52° C. Six l. of the condensed product were adjusted to pH 4.7 with 10 *N* H_2SO_4, and 2,900 ml. of whey were recovered by centrifuging the coagulated mixture. The whey was acidified further to pH 2.0, divided equally and placed in two continuous liquid-liquid extractors[2] each having 2-l. capacity. The remaining volume in the extractors, after adding the whey, was filled with 250 ml. of water and approximately 250 ml. of ethyl ether. Continuous ether extraction of the whey was conducted for 24 hours, after which the receivers were removed from the apparatus, and their contents were combined. During the extraction some emulsion had formed and overflowed with the ether into the receivers. Therefore, the combined contents of the receivers were saturated with NaCl to induce breaking of the emulsion and solution of hippuric acid in the ether phase. The ether layer was separated from the salt solution and then evaporated on a steam bath to about 0.5 ml. of viscous residue. This residue was placed in a refrigerator (4° C.) for 4 days, during which time it changed into a crystalline mass. The mass was extracted with two 25-ml. portions of petroleum ether to remove any lipid material. The crystals then were suspended in 10 ml. of cold 10-per cent HCl, recovered on a filter, and dried under vacuum

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[2] Ace Glass Co., Vineland, N. J.

for 4 hours at room temperature, yielding 130 mg. of crude product. In order to obtain a sufficient quantity of the compound to enable identification, several recovery experiments, similar to that described, were required. The material was recrystallized from a few milliliters of hot water. The habit of the resulting crystals was in keeping with that depicted for hippuric acid (3). Melting points, all by capillary tube, were as follows: Recrystallized product from milk, 186-188° C.; an authentic sample of hippuric acid (Eastman Kodak Co.), 187-188° C.; a mixture of the two, 186-188° C. The p-nitrobenzyl esters of the product from milk and authentic hippuric acid were prepared and observed to melt at 137-138° C., both separately and when mixed. Permanganate oxidation of the unknown yielded benzoic acid, melting with sublimation at 121° C. Determination of the neutral equivalent was made on 4.5 mg. of the product from milk. The value obtained, 176, agrees reasonably well with that of hippuric acid, 179 (7). Results of elemental analyses were as follows: theory for $C_9H_9O_3N$, C–60.33, H–5.06, N–7.82 per cent; found, C–59.84, H–5.02, N–7.62 per cent. These data adequately establish hippuric acid as a constituent of the milk samples studied. However, they afford little information concerning the frequency or concentration with which the compound occurs in mixed herd milks over a period of time.

A method for measuring hippuric acid in milk. Hippuric acid does not lend itself well to quantitative measurement as it occurs in milk. As far as is known, the compound gives no colorimetric reactions which are readily adaptable to quantitative measurement. Considerable effort was expended on the procedure of hydrolyzing hippuric acid and measuring liberated glycine by the ninhydrin reaction. Reproducible results could not be obtained. Hippuric acid is quite resistant to hydrolysis, and the high concentration of acid required for this purpose interfered with the ninhydrin reaction even when careful neutralization was employed. A satisfactory method, based on that of Griffith (2) for measuring the hippuric acid in urine, was devised. The method is as follows: Approximately 1,600 ml. of skimmilk are adjusted to 45° C. Nine g. of trypsin (Nutrional Biochemicals Corp. 1-300 grade) are thoroughly dispersed in about 50 ml. of the milk. This mixture is quantitatively transferred to the sample, which then is digested for 1 hour at 45° C. Under these conditions, protein nitrogen is converted to nonprotein nitrogen. Ten ml. of 70 per cent sulfuric acid are added to the sample with agitation. This acidification precipitates the trypsin and renders hippuric acid more readily extractable with ether. After standing in the cold (4° C.) overnight, the acidified sample is filtered in the cold through Balston No. 14 paper. About the first 100 ml. through the paper is cloudy and should be poured back to refilter. In order to discourage emulsion formation in subsequent ether extraction, a clear filtrate should be obtained. A 1,500-ml. portion of filtrate is transferred to a continuous liquid-liquid extractor of 2-l. capacity. Two hundred ml. of water and sufficient pure ethyl ether are added to bring the level of liquid in the extractor even with the overflow arm. The receiving flask is filled with ether and the apparatus is prepared for operation. At this laboratory, cycling of the ether was effected from a 1-l.

round-bottom flask, the contents of which were heated with a Glas-Col mantle. A Powerstat transformer at a setting of 35 was employed to reduce the power from a 115 V. line. Under these conditions, it was observed that a period of 50 hours of continuous extraction was required to recover quantitatively 50 mg. of hippuric acid from 1,700 ml. of distilled water. These conditions of extraction were employed throughout this study. When extraction is complete, the contents of the receiver are transferred to a Kjeldahl flask. The ether is evaporated on a steam bath and nitrogen in the residue determined in the conventional manner. Milligrams of nitrogen may be converted to milligrams of hippuric acid per 100 ml. of skimmilk by means of the following equation:

$$\frac{\text{mg. of } N_2 \times 12.86}{15} = \text{mg. of hippuric acid/100 ml.}$$

In addition to the conversion factor, the value 12.86 also contains a correction for dilution of the sample with sulfuric acid. No correction was considered necessary for the addition of trypsin, since this is removed when the acidified sample is filtered. The method and results obtained with it are based on the analysis of skimmilk. Control of milk fat as a variable would be essential in any case. However, conversion of values to a whole milk basis by correcting for fat content should be feasible.

Control measures observed in development of the method are worthy of comment. The Kjeldahl procedure measures all of the nitrogen in hippuric acid (5). Twenty-mg. samples of the acid were analyzed in duplicate and yielded 1.57 mg. of nitrogen (theory 1.56 mg.). That the extraction procedure recovers all of the hippuric acid present as such in the digested milk was demonstrated in two ways. Two samples of the same skimmilk were digested and prepared for extraction in the manner described. To one sample 94.9 mg. of hippuric acid were added. After extraction and analysis, it was found that the sample with acid added and its control contained 166.5 and 71.8 mg. of hippuric acid, respectively. The per cent recovery was 98.7. In another experiment, two samples of the same milk were prepared and extracted. One was analyzed for hippuric acid; quantitative recovery of the compound was attempted with the other. The crystals, which formed in the ether extract residue after it had stood for 1 week at 4° C., were extracted with two 10-ml. portions of petroleum ether and then suspended in 10 ml. of cold 10-per cent HCl. This suspension was filtered immediately and dried at room temperature under a pressure of 15 mm. of Hg. until constant weight was obtained. The yield by analysis was 81.1 mg. The weight of crystals recovered was 83.7 mg. These crystals had a melting range of 184-187° C., indicating that they were reasonably pure hippuric acid.

Griffith's method for determining hippuric acid in urine (2) recommends oxidation of the ether extract residue with NaOBr, the purpose being to remove any urea which might be recovered in the extraction. This step has been found unnecessary in the method as applied to milk. The ether extract residue from two samples of the same milk, one untreated and the other treated with NaOBr, gave identical results, 5.5 mg. of hippuric acid/100 ml. of skimmilk. This appears logical since urea and amino acids are probably present in the

digested, acidified milk as H_2SO_4 complexes which are not extracted from dilute aqueous solution with ethyl ether. Phospholipids, which might be a source of error are removed during filtration of the sample. In any event, no evidence was obtained that any form of nitrogen other than hippuric acid is recovered by the extraction procedure.

The biggest difficulty encountered in development of the method concerned preparation of the milk in a form which would not yield a troublesome emulsion during extraction. Several methods of removing the proteins from skimmilk were tried and found unsuitable. In nearly all instances, skimmilk samples which first were digested and then acidified and filtered as described could be extracted satisfactorily. It is conceivable that some of the hippuric acid in milk may be combined with milk protein. The tryptic digestion procedure, used to prepare samples for ether extraction, might be expected to liberate the compound from such a combination. The following experiment was conducted to investigate this point. A sample of skimmilk was divided into two portions. One portion was prepared for ether extraction by tryptic digestion. The second portion was prepared by removing essentially all of the protein. This involved removal of casein by rennet coagulation, and heat coagulable proteins from the rennet whey by pH adjustment, heat treatment, and centrifugation. The hippuric acid content, 3.8 mg./100 ml. of extracted material, was observed to be the same for both preparations. These findings indicate that the method recovers only uncombined hippuric acid. They do not preclude the possibility that hippuric acid may be present in milk in a bound form that is not liberated by trypsin.

The hippuric acid content of skimmilk. At intervals during the period July 1952 to January 1953, 14 samples of skimmilk were analyzed for hippuric acid. The individual values of the samples as related to the dates of production are presented in Table 1. No clear cut seasonal trend is evident in these data. The variations might best be attributed to changes in feed.

DISCUSSION

The animal body disposes of benzoic acid, which is toxic, by uniting it with glycine to form hippuric acid. This product is readily eliminated in the urine. Maynard (6) reports that the ration of herbivorus animals normally contains much larger amounts of hippuric acid precursors than does that of Omnivora

TABLE 1

The hippuric acid content of skimmilk[a] (Mg. / 100 ml. skimmilk)

Sample No.	Date of production	Hippuric acid	Sample No.	Date of production	Hippuric acid
1	7/31/52	6.4	8	11/1	5.5
2	8/13	5.7	9	11/7	4.7
3	9/15	3.1	10	11/14	4.6
4	9/26	5.5	11	11/24	4.7
5	10/3	5.4	12	12/12	5.5
6	10/14	6.2	13	12/22	3.8
7	10/24	5.3	14	1/19/53	4.8
Av.	.....	.....	....	....	5.1

[a] Separated from mixed herd raw milk.

and that in all species there are considerable variations in hippuric acid excretion according to the nature of the feed. Hippuric acid in the urine of dairy cows may range from 0.01 to 4.15 per cent and average 1.17 per cent (4). As is true of a number of other substances which occur in the blood and urine of the cow, hippuric acid finds its way into the milk. In the analysis of milk for nitrogen distribution, it is probable that hippuric acid is determined as nonprotein nitrogen. Another little-known component of the nonprotein nitrogen fraction is orotic acid (1, 9). Recent studies have indicated it to be of nutritional importance (8, 9).

SUMMARY

Hippuric acid was identified as a constituent of the nonprotein nitrogen fraction of milk. A method of measuring the hippuric acid content of milk was developed. This method is based on continuous ether extraction of tryptic digested, acidified skimmilk and measurement of extracted nitrogen by the Kjeldahl procedure. The amounts of hippuric acid in 14 samples of skimmilk, gathered during the period July to January, were determined. These values ranged from 3.1 to 6.4 mg./100 ml. of skimmilk and averaged 5.1 mg./100 ml.

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REFERENCES

(1) Biscaro, G. and Belloni, E. Über einen neuen bestandeil der milch. Chem. Zentr., **2**: 63-64. 1905.

(2) Griffith, W. H. Benzoylated Amino Acids in the Animal Organism. J. Biol. Chem., **69**: 197-208. 1926.

(3) Hawk, P. B., Oser, B. L., and Summerson, W. H. *Practical Physiological Chemistry.* 12th ed., p. 737. The Blakiston Co., Philadelphia. 1947.

(4) Healy, D. J. The Normal Clinical Urinalysis of the Dairy Cow. Am. Vet. Rev., **42**: 184-191. 1912.

(5) Karabinos, J. V. and Dittiner, K. Presence of Hippuric Acid in Milk. Proc. Soc. Exptl. Biol. Med., **53**: 111-112. 1943.

(6) Maynard, L. A. *Animal Nutrition.* p. 95. McGraw-Hill Book Co., New York. 1937.

(7) McIlvain, S. M. *The Characterization of Organic Compounds.* pp. 185-187. The Macmillan Co., New York. 1947.

(8) Reichard, P. The Function of Orotic Acid in the Biogenesis of Pyrimidines in Slices from Regenerating Liver. J. Biol. Chem., **197**: 391-398. 1952.

(9) Wieland, O. P., Avener, J., Boggiano, E. M., Bohonas, N., Hutchings, B. L., and Williams, J. H. Orotic Acid in the Nutrition of a Strain of Lactobacillus Bulgaricus. J. Biol. Chem., **186**: 737-742. 1950.

FREE FATTY ACID CONTENT OF ICE CREAM WITH CERTAIN OBSERVATIONS ON FLAVOR RELATIONSHIP [1]

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Much attention has been given to the action of normal milk lipase in milk, and investigations have been conducted with the view of establishing the fatty acid-rancid flavor threshold level (3, 4, 8). Also, the concentration of free acids in butter and cheese has been related to flavor characteristics of these products (7, 9).

Lipase action in relationship to ice cream quality has not been given specific attention. Such consideration seems justified for three reasons. First, rancid and closely related flavors are found in ice cream. This indicates that under certain conditions of processing and manufacturing, or through improper control of the raw dairy products used, a sufficient quantity of free fatty acids is developed to produce the "off" flavor. This matter becomes increasingly important as greater use is made of low-heat condensed or concentrated milk products as a source of the milk solids. In certain of these products sufficient active lipase may remain to create a flavor problem unless proper precautions are used in processing. Second, free fatty acids when added to the mix have been found to be related to shrinkage in ice cream (2). However, no studies have been made of the relationship of shrinkage to the free fatty acid content of ice cream resulting from normal lipase action. Third, a certain concentration of free fatty acids has been found to be beneficial in improving the flavor of milk chocolate (5). The possibility exists that controlled lipolysis may be utilized by the ice cream maker in the production of a chocolate ice cream with higher consumer acceptance.

Because of the lack of information in this connection, a study was conducted for the purpose of determining the normal free fatty acid content of commercial ice cream and establishing the fatty acid-rancid flavor threshold level in vanilla and chocolate ice creams.

EXPERIMENTAL PROCEDURE

The free fatty acid content of ice cream was determined by a modification of the solvent extraction procedure of Johnson and Gould (6), as follows: *(a)* Thirty g. of melted ice cream mix were weighed into a 250-ml. centrifuge bottle; the mix was diluted with 10 ml. of distilled water and the pH adjusted to 2.0 with 1 *N* H_2SO_4; *(b)* the acidified mix was shaken vigorously with 40 ml. of 95 per cent ethanol for 30 seconds and allowed to stand for 5 minutes; *(c)* sixty ml.

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of ethyl ether and 80 ml. of Skellysolve F were added and the mixture was shaken for 40 seconds; *(d)* the bottle was centrifuged for 10 minutes at 2,000 r.p.m.; *(e)* the solvent-fat layer was transferred to a Mojonnier fat dish by siphoning; and *(f)* the extracted fat was freed from solvents by allowing the pans to stand at room temperature overnight or by using a 90° C. hot plate until bubbling ceased. The last traces of solvent were removed by placing the fat in a 60° F. vacuum oven at 50 mm. mercury for 10 minutes.

The free fatty acid content of the extracted fat was determined by the method of Breazeale and Bird, slightly modified (1). One g. of fat was dissolved in 5 ml. of absolute ethanol and 15 ml. of petroleum ether. Ten drops of an absolute alcoholic phenolphthalein solution (1 per cent) was added, and the solution was titrated to the first definite color change with 0.01 *N* KOH in absolute alcohol using a 5-ml. micro-burette. Results of the titrations are expressed in terms of acid degree: milliliters of $1/N$ base needed to titrate 100 g. of fat. Values for this modification using 1 g. of fat were within $\pm$ 5 per cent of the values obtained for 5 g. of fat by the method of Johnson and Gould (6).

EXPERIMENTAL RESULTS

Fatty acid-rancid flavor threshold level. Laboratory studies were conducted to establish the relationship between the fatty acid level and rancid flavor. In the first series, the experiment involved unflavored plain mixes which contained 10 per cent fat, 10 per cent serum solids, 15 per cent sugar, and 0.3 per cent stabilizer. In the first two trials, raw cream was allowed to become rancid at 40° F., the lipolytic action being accelerated by homogenization. After sufficient lipolysis had occurred, the lipase was inactivated by pasteurization. The mix then was prepared using this lipolyzed cream as a source of fat. In the last two trials, the mix was prepared from raw milk ingredients, and the mix then was homogenized at 2,500 p.s.i., stored at 35° F. until rancid, heated to inactivate the enzyme action, and adjusted with various amounts of the control mix to give mixes of varying acid degrees. These unfrozen mixes, code-numbered to prevent identification by the judges, were scored for flavor by a panel of six experienced judges. The results obtained indicated that the method of preparing the rancid mix did not influence the flavor. Therefore, results for all 30 samples are summarized in Table 1.

TABLE 1

Relationship of free fatty acid content to rancid flavor in experimental plain unfrozen mix.

Average acid degree	Acid degree range	No. of samples	Intensity of rancid flavor[a]	No. of judges detecting rancidity
1.3	0.00-2.00	2	0.00	0
3.04	2.00-4.00	10	0.30	2
4.32	4.01-5.00	4	0.90	5
5.60	5.01-6.00	6	1.80	6
6.33	6.01-8.00	4	2.65	6
9.23	8.01-10.00	3	3.20	6
10.92	10.01-12.00	1	4.00	6

[a] 0 = no rancidity, 1.0 = sl. rancid, 2.0 = rancid, 3.0 = definite rancid, 4.0 = pronounced rancid. Numbers given are averages of flavor designation by six judges.

Data reveal that the rancid flavor was not detected consistently in the plain mix until the acid degree approached the range of 3.5 to 4.5, and the flavor defect was not detected by all judges until the mix had an acid degree in excess of 5.0.

An investigation was made also to determine the relationship of free fatty acids to rancid flavor in chocolate ice cream mix. A homogenized raw mix was prepared with the same composition as the plain mix. The mix was divided into three parts; one part served to provide a plain mix for comparison; a second part was flavored with domestic cocoa; and the third part was flavored with a Dutch process cocoa. Three per cent chocolate and an additional 2.0 per cent sugar were added to the plain mixes to prepare the chocolate flavored product. The mixes were homogenized and the control lot of each series was pasteurized immediately. The remainder of each mix was allowed to become rancid at 40° F. The lipolytic action was stopped by pasteurization, and mixes were adjusted with the control mix to contain various concentrations of free fatty acids.

The unfrozen mixes were judged by the panel to determine the intensity of rancid flavor. Results are presented in Table 2. Whereas all the judges

TABLE 2

Relationship of free fatty acid content to rancid flavor in experimental chocolate mixes

Acid degree	Cocoa	Intensity of rancid flavor[a]	No. of judges detecting rancidity
2.47	None	0.00	0
4.67	None	1.58	6
4.96	None	1.20	6
6.33	None	3.30	6
6.45	None	2.60	6
2.11	Domestic	0.00	0
2.75	Domestic	0.00	0
4.72	Domestic	0.42	3
4.76	Domestic	0.70	4
6.39	Domestic	1.80	6
6.99	Domestic	1.58	6
2.42	Dutch	0.00	0
4.34	Dutch	0.60	3
4.44	Dutch	0.70	4
6.35	Dutch	1.60	6
6.39	Dutch	1.80	6

[a] See footnote Table 1.

detected rancidity in the plain mixes having an acid degree of about 4.7, only three judges detected rancidity in chocolate mix with this acid degree. However, all judges detected rancidity in chocolate mix with an acid degree above 6.3, but the intensity of flavor was much less than in plain ice cream mix with the same acid degree. There was no discernible difference between the samples containing the domestic or the Dutch cocoas from the standpoint of acid degrees and flavor relationship.

Acid degree values of commercial vanilla and chocolate ice cream. Vanilla and chocolate commercial ice creams manufactured by ten different concerns were obtained periodically over a 10-month period from commercial outlets

and were subjected to analysis for free fatty acid concentration. The results are presented in Tables 3 and 4.

All of the commercial samples were judged organoleptically by a panel of three judges. The flavor scores and flavor defects could not be related to the fatty acid values, except that the better ice creams generally contained less free fatty acids.

The data in Table 3 for vanilla ice cream reveal wide variations in acid degrees for individual samples of ice cream. The acid degree values varied from 1.33 to 6.24. Twenty-five per cent of the samples had acid degrees below 2.0, 42 per cent between 2 and 3, 17.5 per cent between 3 and 4, and 15 per cent had acid degrees of more than 4.0.

The four samples of ice cream from some firms were consistently low in free fatty acid content, whereas ice cream from other companies varied widely in

TABLE 3

Free fatty acid content of commercial vanilla ice cream

Firm No.	*Months samples obtained*				
	Aug.	Nov.	Jan.	June	Av.
	Acid degree				
	(ml. N KOH/100 g. fat)				
1	1.33	2.05	1.34	2.26	1.74
2	1.75	1.97	2.12	1.89	1.93
3	1.75	2.04	2.15	1.90	1.96
4	1.76	2.43	2.81	2.37	2.34
5	1.99	2.06	6.24	2.72	3.25
6	2.89	2.14	3.72	5.61	3.59
7	2.91	2.21	2.82	1.97	2.48
8	3.42	3.94	5.71	3.15	4.05
9	3.50	5.14	4.58	2.04	3.81
10	5.40	3.55	2.00	3.61	3.64
Av.	2.67	2.75	3.35	2.85	

TABLE 4

Free fatty acid content of commercial chocolate ice cream

Firm No.	*Months samples obtained*				
	Aug.	Nov.	Jan.	June	Av.
	Acid degree				
	(Ml. N KOH/100 g. fat)				
1	1.26	2.24	1.81	2.38	1.92
2	1.64	2.47	2.33	2.16	2.15
3	2.60	2.15	2.30	2.42	2.37
4	2.25	4.72	3.63	3.41	3.50
5	2.90	2.99	5.83	2.51	3.44
6	3.14	2.66	4.06	4.34	3.55
7	3.26	2.61	3.13	3.51	3.13
8	3.06	3.44	3.66	2.62	3.19
9	2.25	3.04	3.13	2.96	2.84
10	4.86	4.29	3.57	3.46	4.04
Av.	2.75	3.06	3.34	2.97	

fatty acid content. Also, company 8 and, to some extent, companies 9 and 10, manufactured ice cream in which the free fatty acid content was, as a rule, much higher than that in ice cream manufactured by the other firms. There was a trend for the winter ice creams to have slightly higher acid degrees than the summer samples.

The wide variations in the fatty acid content of the ice creams indicate that in certain cases (those in which the fatty acid level was consistently high), either raw products were used which contained an appreciable quantity of free fatty acids, or certain processing steps were involved which favored lipase action prior to pasteurization. For example, investigation revealed that in one plant the high free fatty acid content of the ice cream appeared to be related to the use of insufficient heat to inactivate the enzyme prior to subjecting the milk ingredients to condensing.

The average acid degrees for the chocolate ice cream (Table 4) were not appreciably different from the average acid degrees of the vanilla samples. However, there was no significant consistent seasonal variation in acid degrees in the chocolate samples, and there were fewer chocolate than vanilla ice creams with acid degrees below 2.5 (13 chocolate as compared to 22 vanilla ice creams).

Results in Tables 3 and 4 reveal that the acid degrees of certain commercial ice creams were above the level which had been found earlier to cause rancidity in experimental mixes. This was more often true for vanilla ice cream than for chocolate. Only two chocolate samples exceeded the threshold acid degree range of 4.7 to 5.0, whereas six vanilla ice creams exceeded the threshold acid degree of approximately 4.0 to 4.5.

DISCUSSION

On the basis of the data presented, it appears that the free fatty acid content of ice cream merits attention. The fatty acid-rancid flavor threshold in ice cream is more than twice the threshold value of 2.0 established for milk or cream (3, 4). This indicates that the other components in ice cream obscure the rancidity and make difficult its detection by organoleptic means. The obscuring effect of chocolate is noticeable, since free fatty acid level for detectable rancidity in chocolate ice cream was appreciably greater than that for vanilla.

The acid degree of strictly fresh ice cream appears to be definitely higher than the free fatty acid content of the fresh milk ingredients used in its manufacture. Plain mixes made as previously described had an acid degree of 2.3-2.5, whereas the fresh dairy products used in the mixes averaged an acid degree of 0.9. Non-dairy products may contribute to the acid degree of ice cream under certain conditions.

The wide variations in the free fatty acid content of the commercial samples may be related, in certain instances, to differences in manufacturing practices. Furthermore, the wide and rather spasmodic variations in the free fatty acid content may indicate why ice cream plants experience unexpected problems with such quality defects as shrinkage.

The commercial sample results indicate that the free fatty acid values are

sufficiently high in certain cases as to be a factor in affecting the flavor of ice cream. However, there may not always be a fixed relationship between flavor and the free fatty acid content, since the type of free fatty acid is an important factor, and the means by which the fat splitting takes place will affect the type of free fatty acid produced. This was revealed by observations in which homogenized raw mix with an acid degree of 4.4 was judged to have an intensity of rancidity of 1.0, whereas a mix made rancid with a commercial lipase preparation had an acid degree of 4.4 and a rancid flavor intensity of 3.0. Thus the variation in the types of acids formed by lipases in experimental and commercial conditions might explain the lack of rancid flavor in the vanilla samples. A second possibility is that the vanilla may mask partially the rancid flavor.

The fact that the acid degree values of many chocolate ice creams were just below the threshold level of rancidity poses an interesting question as to whether or not this small concentration of free acids may be beneficial to the flavor of the ice cream. If so, then a study should be made to establish the type and degree of lipolysis which is desired, so that a uniform product can be manufactured.

SUMMARY

Studies were conducted on vanilla and chocolate ice cream mixes to establish the rancid flavor-free fatty acid threshold level. Results revealed that rancidity was detected in plain ice cream mix at an acid degree of approximately 4.0 to 4.5 and in chocolate ice cream mix at acid degrees between 5 and 6. These values are considerably higher than the threshold levels which have been reported for milk and cream.

The free fatty acid content of fresh ice cream was definitely higher than the free fatty acid content of the fresh milk and cream constituents.

Commercial vanilla and chocolate ice creams were obtained from 10 different manufacturers at four different periods during the year and analyzed for free fatty acids. The samples varied widely in free fatty acid content, ranging from 1.33 to 6.24 for vanilla and from 1.26 to 5.83 for chocolate. Ice creams from certain manufacturing plants were consistently low in free fatty acid content, whereas ice creams from other plants were consistently high or erratic in the concentration of free fatty acids. The wide variations in free fatty acids may be related to certain defects in commercial ice creams.

REFERENCES

(1) Breazeale, D. F., and Bird, E. W. A Study of the Methods for the Determination of Acidity in Butterfat. J. Dairy Sci., 21: 335-344. 1938.

(2) Dahle, C. D., and Lando, J. C. Report on Shrinkage. Ice Cream Rev., 34, 6: 50, 52, 66, 68-72. 1951.

(3) Dunkley, W. L. Hydrolytic Rancidity in Milk. I. Surface Tension and Fat Acidity as Measures of Rancidity. J. Dairy Sci., 34: 515-520. 1951.

(4) Gould, I. A. Relationship of Fat Acidity to Rancidity in Homogenized Raw Milk. J. Dairy Sci., 27: 167-172. 1944.

(5) Hollender, H. A. Special Milk Powders for the Maufacture of Milk Chocolate. Ph.D. Dissertation, Univ. of Wis. Unpublished, 1948.

(6) JOHNSON, B. C., AND GOULD, I. A. Milk Lipase System. III. Further Studies of the Solvent Extraction Procedure for Obtaining Fat from Milk for Titration. J. Dairy Sci., 32: 447-451. 1949.

(7) KOSIKOWSKY, F. V., DAHLBERG, A. C., GUTHRIE, E. S., AND KNAYSI, G. The Acid Degree, Free Volatile Fatty Acids and the Flavor Score of Salted Commercial Butters. J. Dairy Sci., 30: 211-222. 1947.

(8) KRUKOVSKY, J. N., AND HERRINGTON, B. L. Studies of Lipase Action. VI. The Effect of Lipolysis upon the Flavor Score of Milk. J. Dairy Sci., 25: 237-239. 1942.

(9) LANE, C. B., AND HAMMER, B. W. Bacteriology of Cheese. IV. Relationship of Fat Hydrolysis to the Ripening of Cheddar Cheese. Iowa Agr. Expt. Sta. Research Bull. 291. 1941.

UREA AS A PROTEIN REPLACEMENT FOR RUMINANTS: A REVIEW

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It has been recognized for some time that urea (and other nonprotein nitrogen-containing compounds) may be used to replace a portion of the protein in the rations of ruminant animals (28, 104). The mechanism making this possible appears to be one in which proteins are synthesized from simple nitrogenous substances by the growth and multiplication of the bacteria of the rumen followed by the digestion of these protein-containing bacteria farther down the digestive tract of the host animal. As recently as 1937, it was not commonly accepted that urea is converted to proteins in amounts of any great significance to ruminants. It was suggested that the apparent protein-sparing action of urea may have been the result of such mechanisms as a neutralization by ammonia of organic acids formed in the digestive tract of the ruminants (42). The results of more recent experiments in which nitrogen balance (25, 30, 31, 44, 49), growth (9, 31, 33, 62, 71, 102), milk yield (1, 67, 80), and body composition (31, 33, 49, 91) were examined suggested that urea is converted to protein. In other experiments (32, 59, 60, 94) the decreased concentration of ammonia and the increased level of protein in rumen ingesta were considered as evidence of the conversion of ammonia nitrogen to protein nitrogen. As pointed out by McNaught and Smith (54) and Pearson and Smith (68), this kind of evidence is subject to criticism in that portions of the ingesta are being transferred constantly from the rumen to other parts of the digestive tract or are being absorbed into the blood, effecting a disappearance which is difficult to interpret. In addition, ingesta sampling difficulties, differences in the solid and liquid portions of ingesta, amount of water consumed and saliva secreted, the washing out of various nutrients at rates different from those of urea, and the ammonia resulting from the hydrolysis of true protein obscure the interpretation. More direct evidence supporting the earlier findings was reported recently by Watson *et al.* (90). Nitrogen-low diets supplemented with urea labeled with N^{15} were fed to sheep for 4 days, after which time they were slaughtered and the quantities of N^{15} in the protein and nonprotein fractions of the kidneys, liver, and blood were measured. Since the quantity of N^{15} in these body proteins exceeded that found in the body proteins of similar sheep fed unlabeled urea, it was concluded that urea nitrogen is utilized in the formation of body proteins by ruminants (90).

Under certain conditions, the supply of conventional protein supplements is inadequate and, generally, protein is the most expensive portion of the animal ration. As a consequence, this review will deal with some of the problems of

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feeding urea to ruminants with special attention being given to the results of experiments conducted during recent years.

FACTORS INFLUENCING THE UTILIZATION OF UREA BY RUMINANTS

The use of urea and other nonprotein nitrogenous compounds in the rations of ruminants has been examined experimentally from the standpoint of the value of these substances for growth, lactation, and meat and wool production. Although the degree of response measured in some of the experiments reported is variable, it is clear that, under certain conditions of feeding, urea has considerable value as a protein replacement. The amount of urea which can be utilized by ruminant animals, or the amount of protein which can be replaced by urea, has been found to depend to a large extent upon the following factors: *(a)* amount and nature of true protein contributed by the ingredients of the ration, *(b)* amount and kinds of carbohydrates in the rations, and *(c)* level of urea causing toxicity. Other factors, such as age of the animal, the amounts of certain amino acids, mineral elements, and roughages and concentrates in the ration, and the length of the feeding period may have varying effects upon the utilization of urea by certain ruminant species and may explain some of the variations in the responses observed.

Effect of protein. According to Hart *et al.* (33) and others, the ability of ruminant animals to utilize simple nitrogenous compounds as a source of protein is explainable by the synthesizing ability of the microorganisms of the forestomachs. The activity of the microorganisms depends upon the kind of ration fed to the host animal. As a result of experiments conducted in vitro, Wisconsin workers (92) found that the level of protein in a medium inoculated with rumen microorganisms influenced the rate of conversion as well as the amount of urea converted to protein. As the concentration of protein (casein) in the "artificial" rumen was increased, the amount of urea converted to protein decreased. It became necessary, however, to examine this problem under animal-feeding conditions. This was studied by determining the concentrations of protein, total nonprotein and ammonia nitrogen in the rumen ingesta of a cow fed a ration of 15 lb. corn silage, 4 lb. timothy hay, and 4 lb. concentrates (94). The basal concentrate mixture consisted of 50% ground corn and 50% ground oats. The proportion of protein equivalent [1] in the concentrate feed was varied from trial to trial by the addition of various quantities of linseed oil meal and/or urea. Although it was found that some urea was converted to protein when the crude protein [2] content of the basal concentrate mixture supplemented with linseed meal was 20 to 24%, the utilization of urea nitrogen decreased decidedly as the crude

[1] The term "protein equivalent" used in this review with reference to urea-containing feeds means percentage of total nitrogen $\times$ 6.25. This usage should not be confused with the British usage in which protein equivalent $= \frac{\text{digestible crude protein} + \text{digestible true protein.}}{2}$

[2] The term "crude protein" has been employed in this review to refer to the percentage of total nitrogen $\times$ 6.25 in common feedstuffs.

protein (provided by linseed meal) content of the concentrate mixture was increased above 18%. When the basal grain mixture (50% corn and 50% oats) was not supplemented with linseed meal, increasing additions of urea to a level of 4.5% (or a protein equivalent of approximately 12%) were utilized efficiently. This combination of urea, corn, and oats provided a concentrate mixture containing approximately 23% of protein equivalent.

As a result of an earlier experiment, Wegner *et al.* (93) reported that when a ration of which urea composed 1 to 5% of the dry matter was ingested, urea disappeared from the rumen within 4 to 6 hours. This was presented as partial evidence for the conversion of urea nitrogen to protein. An experiment conducted by Pearson and Smith (68) in much the same manner as those conducted by Wegner *et al.* (93, 94) did not allow the synthesis of protein from urea to be demonstrated. Pearson and Smith pointed out a variety of difficulties which preclude definite conclusions based upon the changing concentrations of nonprotein and protein nitrogen in ruminal ingesta and, upon reanalyzing the data of Wegner *et al.*, concluded that definite proof of protein synthesis was not obtained in these experiments.

Pearson and Smith (70) found that certain amino acids promoted, and others depressed, the in vitro synthesis of protein from urea by microorganisms of the rumen. Using a somewhat similar technique, though employing a much longer incubation period, Burroughs *et al.* (17) examined the effects of proteins of different amino-acid composition upon the conversion of urea nitrogen to protein nitrogen. The results were interpreted to mean that the nitrogen required by ruminal microorganisms is in the form of ammonia and that more complex nitrogenous compounds such as amino acids are not required. However, a marked improvement in the retention of nitrogen by lambs has resulted from the supplementation of urea-containing rations with methionine (44, 48).

The addition of urea to basal rations (7.8% of crude protein) containing either cottonseed meal, soybean oil meal, or corn gluten meal to form rations containing 10.6% of crude protein resulted in the same amounts of nitrogen being retained by lambs (26). Without urea supplementation, the amounts of nitrogen retained by lambs consuming the three basal diets also were similar.

As a result of experiments conducted in vitro by Pearson and Smith (70), McNaught and Smith (54) suggested that the nature of the protein in a ration may affect the efficiency with which nonprotein nitrogen is utilized as protein. These workers (54, 70) pointed out the possibility that when the ration contains insoluble protein the amount of ammonia formed from the protein may be small, favoring a more efficient utilization of urea. In in vivo experiments, McDonald (52) demonstrated that large amounts of ammonia are formed from casein and gelatin but that the addition of zein caused no change in the ammonia content of the rumen liquor. It is noteworthy that gelatin is soluble in water and readily hydrolyzed by proteinases but that zein is insoluble in water and resistant to proteolysis.

Because of the apparent complexity of the conditions in the rumen and the problems encountered in attempting to simulate these conditions in vitro, it is

difficult to apply the results of experiments conducted in vitro to the feeding of the intact animal. One of the major problems involved in the study of urea conversion by microorganisms in vitro appears to be the maintenance of microorganisms of similar character throughout the incubation period. Since Baker (3, 4) found that ruminal flora and fauna were much different microbiologically after an incubation period of even one day from those examined prior to incubation, results obtained in vitro could be quite misleading from the standpoint of application to feeding practice.

In many of the early experiments the poor utilization of urea is attributable to the relatively high level of true protein in the rations to which urea was added. For concentrate feeds containing 9 to 12% or less of crude protein, no more than 5 to 8% of protein equivalent (1.9 to 3.0% of urea containing 42% of nitrogen) should be provided as urea because of inefficient usage above this level.

Effect of carbohydrates. The influence of carbohydrates on the conversion of urea nitrogen to protein was studied (59) by measuring the amounts of protein and ammonia nitrogen in the rumen ingesta at different times after the feeding of various rations. When timothy hay was fed with urea, the level of protein in rumen ingesta was the same as that when the hay was fed alone or in combination with starch. However, the amount of ammonia nitrogen in the ingesta was higher when timothy hay and urea were fed than when timothy hay was fed alone or with starch. These findings suggested that timothy hay alone did not provide a suitable medium for the bacterial synthesis of protein from urea. The addition of starch to the timothy hay-urea ration resulted in an increase in the protein content of the rumen ingesta. McDonald (52) reported that the addition of starch to the rumen of sheep 20 hours after consuming a zein-containing diet (at which time the ammonia content of the ingesta was high) resulted in a rapid reduction in the ammonia content. This suggests that starch provided a source of energy needed by microorganisms to utilize ammonia.

In other experiments Wisconsin workers (60) studied the influence of molasses upon the synthesis of protein from urea. Timothy hay alone or in combination with molasses resulted in rumen ingesta containing 6.5 to 7.7% of true protein. The addition of urea to a ration of molasses and timothy hay increased the true protein level of the rumen contents to 9.3%. However, the addition of starch to a ration of timothy hay, molasses, and urea resulted in a further increase in true protein content to 11.0%. Since these results indicated that the addition of starch to the ration promoted protein synthesis to a greater extent than did the molasses, a further experiment (60) was conducted with growing heifers approximately 3.5 months old and weighing 200 lb. A ration containing timothy hay, cane molasses, and urea and providing 11.6% of protein equivalent, of which urea composed 60 to 65% of the total nitrogen, resulted in an average daily gain of 0.72 lb. of body weight in three heifers. When the same ration was supplemented with 0.3 lb. of starch, the daily body weight gain was 1.4 lb. Since the same heifers were employed in both trials, an effect of age upon the gain in weight may have existed. Seven

weeks elapsed between the first and second trials, which were of 19 and 4 weeks duration, respectively. Since Mills *et al.* (60) suspected that the protein synthesized from the hay-molasses-urea ration may have been of poor quality, casein (0.3 lb./day) was added to a ration otherwise of the same composition as that used in the previous trial. This ration, providing 13.5% of protein equivalent, resulted in an average daily gain of 1.6 lb.

The results of these feeding experiments (60), as well as those of the experiment in which ruminal ingesta was studied, indicated that only partial utilization of urea by ruminants takes place when molasses is the main readily available carbohydrate fed and that starch is much more effective than molasses in the conversion of urea to protein. This may mean that the sugars of molasses are absorbed or passed from the rumen or degraded too rapidly to be of much use to the microorganisms. In an experiment (80) with milking cows, the addition of molasses to a ration containing timothy hay, corn silage, ground corn, and oats produced no significant effect upon the utilization of urea nitrogen, as evidenced by the quantity of milk produced. Willett *et al.* (98) found that the substitution of 25% of cane molasses for a portion of the pineapple bran in a ration for milking cows did not affect the utilization of urea, as determined by milk yield. The basal concentrate employed in these experiments also contained 21 to 22% of both soybean meal and barley.

The value of urea as a source of nitrogen in rations containing different high-carbohydrate feeds was studied by Bell *et al.* (10) in a series of digestion and nitrogen-balance trials conducted with steers. The carbohydrate feeds used singly in the rations were ground corn, dehydrated sweet potatoes, cracked milo, rolled barley, cane molasses, and combinations of cane molasses and ground corn. Each of these was combined with prairie hay and sufficient amounts of high-protein supplement to form rations containing 8% crude protein. Urea was added to increase the protein equivalent level to 11%. A greater improvement in nitrogen retention resulted from the addition of urea to the corn-containing ration than from the addition of urea to the ration containing cane molasses. The other carbohydrate sources appeared to be about equal to corn.

Previously, Pope *et al.* (74) found that the improvement in the retention of nitrogen by steers was the same when urea supplemented rations containing 50% of prairie hay and 50% of either corn or molasses or 25% of corn and 25% of molasses.

Certain of the findings in feeding experiments are supported by the results of experiments conducted in vitro by British workers (70, 84). As a result of incubating ruminal ingesta with urea and each of several carbohydrates and other substances, starch was found to be more effective in promoting protein synthesis than glucose, maltose, or galactose (all of which were effective), sucrose (which was partially effective), or dextrin, glycerol, or lactic acid (all of which were relatively ineffective). Also, various decomposition products of carbohydrates were not effective (53). Cellulose added to a synthetic medium was found to be a poor source of energy for protein synthesis (92).

Pearson and Smith (70) demonstrated that protein synthesis from ammonia and the hydrolysis of protein to form ammonia occur simultaneously; however, when starch was present in the medium, the quantity of protein synthesized exceeded that hydrolyzed. These observations largely have been verified by the in vivo experiments conducted by McDonald (52).

In other in vitro experiments Arias *et al.* (2) examined the value of dextrose, sucrose, starch, cellulose, cane molasses, and ground corn cobs as sources of energy for the conversion of urea nitrogen to protein nitrogen. Each source of energy aided urea utilization when cellulose (a component of all basic media used) was digested. However, when relatively large amounts of readily available carbohydrates were added to media, the extent of cellulose digestion and urea utilization was reduced. On the other hand, small amounts of readily available carbohydrate seemed to stimulate the digestion of cellulose and the utilization of urea. Arias *et al.* (2) concluded that urea utilization in vitro is greatest when medium amounts of both readily available carbohydrates and complex carbohydrates are present or when a small quantity of readily available carbohydrates and a large amount of complex carbohydrates are present in the medium.

Although the mechanisms involved in in vitro experiments actually may represent those occurring in vivo (and in the case of the influence of various carbohydrates upon urea utilization, it would appear that the results of animal-feeding experiments bear out at least some of those obtained in vitro), the criticism of in vitro work pointed out in connection with the influence of protein on urea utilization applies in this instance as well. The results of the in vivo experiments (60) in which timothy hay was supplemented with urea suggest that the presence of cellulose and other carbohydrates more complex than starch do not result in appreciable utilization of urea. Perhaps this is explained on the basis that an inadequate amount of cellulose is hydrolyzed sufficiently soon after ingestion to become available at the time when needed by the bacteria. A need for available carbohydrates soon after ingestion of urea was indicated by the work of Lenkeit and Becker (43), Wegner *et al.* (93), and Pearson and Smith (69), who demonstrated that urea is rapidly hydrolyzed. This need appears to be satisfied by starch. When concentrate feeds fed to ruminants contain the usual amounts of cereal grains, a sufficient quantity of starch seems to be provided for satisfactory protein synthesis, provided that other conditions are satisfactory.

Toxicity of urea. The animals employed by Hart *et al.* (33) in studies of growth were slaughtered after having received rations containing urea, ammonium bicarbonate, or casein for a period of 12 months. The kidneys of the animal which received a ration containing 4.3% of urea were hypertrophied and showed hyaline degeneration and casts formed in the tubules. (This animal also had manifested diuresis during the feeding trials.) In addition, necrotic areas were found in the liver, whereas the spleen and muscle tissues appeared to be unaffected. The animal whose ration contained 2.8% of urea showed no pathological alterations of the spleen, liver, and muscle tissues; however, evidence

of a slight, but definite, kidney damage was observed. The cellular structure of the kidneys of the animals receiving rations containing either 1.4% of urea, 11.1% of ammonium bicarbonate, or 11% of casein was normal. A slight congestion, however, was found in the kidneys of animals consuming these rations. Work *et al.* (103) fed fattening steers rations containing as much as 2.29% of urea and did not detect any liver or kidney damage.

As a result of the studies made by the Wisconsin (33) and Hawaiian groups (103), it appeared that a level of urea between 2.3 and 2.8% of the total ration may be harmful to cattle. Harris and Mitchell (31) reported that kidney damage was not evident in wethers fed rations containing 3.15% of urea.

Recently, Dinning *et al.* (20) studied the effects of orally administered urea upon cattle and sheep. It was observed that ataxia, severe tetany, a retarded respiratory rate, and salivation occurred approximately 20 minutes after drenching the cattle with 116 g. or more of urea. No ataxia resulted from dosing cattle with 57 g. The blood levels of urea and ammonia were approximately 16 to 18 and 2 mg. per cent, respectively, at the time that ataxia was noted. As the concentration of ammonia increased, the severity of the tetany became more pronounced. These workers found no symptoms of toxicosis in 8 steers fed daily 200 g. of urea mixed in the ration. They concluded that urea mixed in the feed may be consumed in large quantities without ill effects; however, the same or even a smaller quantity introduced suddenly into the rumen or esophagus may be fatal.

The most extensive study made of acute urea toxicosis in sheep was reported by Clark *et al.* (19). Some of the symptoms described by these workers are: dullness followed by hyper-aesthesia, severe twitching of the musculature over the whole body, and bloat, followed by severe tetanic spasms of the skeletal musculature of the whole body, prostration of the animal with the legs extended and claws abducted, labored breathing, and regurgitation of ruminal contents just prior to death. The clinical symptoms occurred 30 to 60 minutes after administering as little as 10 g. of urea directly into the rumen. These investigators pointed out that the syndrome resembles that of strychnine poisoning. The cause of death was found to be an acute circulatory collapse with generalized venous stasis. In some animals hydrothorax and pulmonary congestion was observed. Hemorrhage under the epi- and endocardium were almost always found. In some cases the liver and spleen were congested and hemorrhagic diathesis was observed in the small intestine. In all animals the liver and kidneys were severely degenerated and, upon microscopic examination, revealed a marked fatty degeneration. It is striking that these lesions were observed in animals which died within one hour after having been given the urea.

After the administration of urea, the motility of the rumen decreased or ceased. This was associated with an increased alkalinity of the rumen contents. The cause of the tetanic spasms and circulatory failure was not ascertained. However, the intravenous injection of large quantities of urea or of ammonium hydroxide did not produce these effects or the lesions described.

Dilute acetic acid administered into the rumen or intravenously was found to be an effective antidote even when the symptoms were advanced.

The toxicity of urea was believed to be modified by the quality of the diet and the activity of the ruminal flora (19). Larger quantities of urea were tolerated by sheep consuming alfalfa hay than by those receiving poor-quality grass hay or by sheep receiving sugar and casein singly in addition to a hay diet. The observation that urea in quantities of 3 to 12% of the medium had a bacteriostatic effect on certain organisms is of interest (95). The possible inhibition of ruminal bacterial growth by urea does not appear to have been examined.

A preliminary study involving 5 steer calves full fed prairie hay revealed that urea fed at levels of 0.02 to 0.06 lb. per 100 lb. of body weight per day caused no ill effects (13). Five additional calves were full fed pellets containing 4% of urea with prairie hay ad libitum during 14 days. A little more than 10 lb. of pellets (0.4 lb. of urea) were consumed per animal daily. The average body weights at the beginning and end of the period were 455 and 469 lb., respectively. After a period of 62 days, during which a standard fattening ration was fed, the animals were slaughtered. Gross and histological examinations showed no evidence of kidney damage.

The feeding of approximately 0.6 lb. of urea daily during a 180-day period caused no ill effects other than diuresis in calves weighing 300 to 500 lb. (62). No evidence of damage to the kidneys or other visceral organs was found. Woodward and Shepherd (101) fed corn silage containing urea to cows and found no evidence of harm on post-mortem examination. For 61 days, a Holstein cow consumed daily 104 lb. of silage containing 1.17 lb. of urea. No abortion or other evidence of toxicosis was found in 10 mature beef cows fed 0.12 lb. of urea per head daily while grazing winter grass (13).

As a result of employing gross and histological examinations of tissues as criteria of toxicosis, Briggs *et al.* (15) concluded that yearling steers refused to eat enough feed containing 8 per cent of urea to suffer adverse effects.

Since the quantities of urea which generally would be used in practice are less than the apparent toxic level and since urea is quite unpalatable, there appears to be no need for great concern in its use, provided that it is uniformily mixed in the feed. Careless mixing, however, may cause serious trouble.

Effect of other factors. Other factors may influence the protein replacement value of urea. The extent of development of the rumen and its microbial population undoubtedly influence an animal's ability to utilize urea. Although the age at which efficient utilization occurs has not been studied extensively, it appears that calves as young as 2 months of age are able to convert a considerable amount of urea nitrogen into protein nitrogen (49).

In their in vitro experiments, Burroughs *et al.* (17) observed that the addition of casein or gelatin to the medium resulted in a greater digestion of cellulose than when urea was the sole source of nitrogen. As a consequence, these workers suggested that these proteins contributed needed mineral elements

such as phosphorus and sulfur. In further experiments, Burroughs *et al.* (18) found that the ash of molasses or of alfalfa, iron, and phosphorus stimulated the utilization of urea in vitro. The need for elements other than these such as sodium, potassium, calcium, magnesium, chlorine, and sulfur (which were components of the artificial saliva) seemed to be indicated.

Thomas *et al.* (88) showed that lambs do not utilize the nitrogen of urea when the diet is deficient in sulfur. Recently, Gallup *et al.* (26) reported on the influence of several factors associated with the ration upon urea utilization by lambs. Three basal rations containing, respectively, 84, 49, and 29% of cottonseed hulls, and ground corn, and corn starch were supplemented with urea to form rations containing approximately 10% of protein equivalent. The digestion coefficients for the nitrogen of these rations were 41.0, 45.4, and 59.6, respectively. Nitrogen retention (0, 5.0, and 21.8% of intake, respectively) also was improved as the amount of hulls in the ration was reduced. Upon substituting 49% of prairie hay for the cottonseed hulls in the second ration, the digestibility of the nitrogen was increased to 61.9%, and 25% of the nitrogen consumed was retained. Although these observations indicate that urea becomes less efficiently utilized as the proportion of roughage in the ration increases, it cannot be stated with certainty that this effect is due to the amount of roughage per se. Since the substitution of cottonseed hulls into the rations used in these experiments (26) was made at the expense of ground corn and corn starch, the poor utilization of urea in the "high-roughage" rations may well have been the result of insufficient quantities of available carbohydrates.

The ease with which urea is dissolved in water and hydrolyzed within the rumen suggests that conditions must be optimal for efficient conversion to protein. Even when conditions are seemingly adequate (based upon present information), there is a considerable wastage of nitrogen. These observations indicate that attention needs to be given to means of extending the nitrogen of urea and, thereby, possibly replacing a greater proportion of dietary protein than is now possible. Whether a less soluble product of urea or of other nonprotein nitrogen compounds or whether urease inhibitors or feeding techniques would result in a more gradual release and a more efficient utilization of urea is not known. An experiment (13) in which steers were fed the same amount of urea per day in two meals daily as was fed in one meal on alternate days is of interest with respect to nitrogen utilization. The nitrogen of urea fed daily in two meals was somewhat more digestible and was stored to a greater extent than that fed in one meal on alternate days. The digestibility and storage of the nitrogen of cottonseed meal studied in the same manner was not affected by the method of feeding.

QUALITY OF PROTEIN SYNTHESIZED FROM UREA

Since the Wisconsin group (33) found that a ration containing yellow corn, starch, timothy hay, and urea and providing a protein equivalent of 9.44% supported fairly good growth of calves, it was indicated that the protein formed from urea was of at least fair quality, although the protein of the

corn may have been an effective supplement. The results of Illinois experiments (31) showed that rations too low in protein to support growth of lambs would promote appreciable growth when urea was added, yet the growth response was not as great as that occurring when casein was fed. Although these findings (31, 33) suggested that the protein formed from urea must be of at least fair quality, the degree of quality remained to be determined.

Biological value of urea nitrogen. Several workers have attempted to measure the biological value of urea nitrogen. In an experiment (32) with calves and growing steers, the biological value of urea nitrogen fed at a level providing 80 to 90% of the total nitrogen of the ration was 34 as compared to 60 for soybean nitrogen. In other experiments in which urea nitrogen composed approximately 80% of the total dietary nitrogen, biological values of approximately 45 to 50 were obtained (29, 36). It is probable that urea fed at very high levels was utilized considerably less efficiently than it would have been at lower levels. When nitrogen was provided by urea or casein at levels just maintaining equilibrium in lambs, the biological values were 62 and 79, respectively (30). The biological values of the nitrogen of a corn-silage ration supplemented with sufficient urea to provide 8, 11, and 15% protein equivalent were 74, 60, and 44, respectively.

A comparison of rations containing 11.6 and 17.0% protein equivalent, of which urea contributed 46 and 63%, respectively, showed that 12.99 and 12.40%, respectively, of the absorbed nitrogen was retained (29). The biological values of the nitrogen of the two rations were: 11.6% of protein equivalent, 47.6, and 17.0% of protein equivalent, 35.8. A series of comparisons between the biological values of the nitrogen of urea and other sources when added to the same basal diet to form rations of 10.5 to 13.0% of protein equivalent were reported by Hamilton *et al.* (29) as follows: urea, 48.8 and nonfat dry milk solids, 52.3; urea, 58.8 and corn gluten feed, 63.8; urea 66.9 and nonfat dry milk solids + cystine, 64.4; urea, 52.7 and linseed meal, 62.6; urea, 48.6 and casein, 48.0; urea, 45.8 and casein + cystine, 54.5 and urea, 45.1 and urea + cystine, 43.4. Only the difference in the biological values of urea and linseed meal was statistically significant.

In a comparison of the utilization of urea nitrogen with that of the nitrogen of wheat gluten by growing steers and lambs, it was found that the efficiency of nitrogen utilization was 50 to 60% for urea and 60 to 63% for wheat gluten (25). Johnson *et al.* (35) demonstrated that, when a basal ration of red top hay, corn, and corn molasses was supplemented at the expense of molasses with sufficient urea, soybean meal, or casein to provide a ration of 12% protein equivalent, the biological value of the nitrogen, regardless of the source, was approximately 60 for growing lambs. This figure agreed well with the biological value of the nitrogen provided by corn silage and urea (31). The biological value of the nitrogen of urea-containing rations appears to be comparable in many cases to that of rations containing 10 to 12% crude protein provided by combinations of corn, timothy hay or corn stover and soybean meal, linseed meal, or corn gluten meal (58).

In later studies of the efficiency with which the nitrogen of various sources is utilized by lambs, Miller and Morrison (57) found no difference in the quality of protein provided by timothy, alfalfa, and mixed timothy-alfalfa hays. Lambs utilized the nitrogen of soybean meal, linseed meal, corn gluten meal, casein, and nonfat dry milk solids equally efficiently. The nitrogen of rations in which urea composed more than one-half of the total nitrogen was used less efficiently than similar rations containing linseed meal or mixtures of linseed meal and smaller proportions of urea. The nitrogen of rations other than those in which urea supplied a large proportion of nitrogen had a biological value of approximately 62, and 32% of the digested nitrogen was stored. The data obtained in 325 nitrogen-balance trials indicated that, for lambs, there is very little difference in the quality of protein provided by most feedstuffs (57).

Influence of sulfur and methionine on utilization of urea nitrogen. Although it was demonstrated (30, 31) that lambs utilize urea nitrogen for maintenance and growth, it was found in experiments conducted at Cornell (99) that for fattening lambs urea did not have as much value as linseed meal as a supplement in a ration consisting of commonly used feeds. Urea employed as the major source of nitrogen resulted in an average daily gain of 0.26 lb. per day whereas linseed meal supplementing the same basal ration gave average daily gains of 0.32 lb. (99). Sodium sulfate was added to some of the rations in an attempt to improve the utilization of urea nitrogen but was without success. Similar observations relative to sulfur supplementation were reported by Nehring and Schramm (66).

Since these findings (99) suggested that urea was inefficiently converted to protein or that the protein synthesized by the microorganisms was of poor quality, Loosli and Harris (48) tested the value of methionine as a supplement to a urea-containing ration. The basal diet employed consisted of the following percentages of various constituents: alfalfa meal, 5; timothy hay, 29; cane molasses, 5; yellow corn, 12; ground oats, 8; brewers' yeast, 0.9; irradiated yeast, 0.1; corn oil, 1.3; minerals, 0.88; and corn starch, 28.3 to 35.5 (depending upon the amounts of the various sources of nitrogen used.) The experimental diets were formed by adding to the basal diet the following percentages of the nitrogen sources tested: linseed meal, 9.54; urea, 1.25; urea, 1.25 + sodium sulfate, 1.06; and urea, 1.02 + methionine, 1.11. The protein equivalent content of the basal diet was 6.55% and that of the other diets ranged from 9.97 to 10.58%. The average daily gains in body weight were: basal, 0.07 lb.; basal + urea, 0.17; basal + urea + sulfate, 0.18; basal + urea + methionine, 0.28; and basal + linseed meal, 0.31. The proportions of the dietary and absorbed nitrogen retained by the body were approximately the same in lambs receiving the basal diet supplemented with linseed meal as in those consuming the basal diet supplemented with urea and methionine. This experiment further showed that the protein formed from urea was inferior to that provided by linseed meal and to that resulting from methionine and urea. It is interesting to note that the addition of sulfate to the urea-containing diet improved the nitrogen balance and the proportion of the dietary nitrogen retained, though

the rate of gain was not affected (48). Recently, Starks *et al.* (85) demonstrated that the nitrogen of urea is retained to a greater extent by lambs consuming rations containing 0.7% of elemental sulfur than by those receiving rations containing 0.06% of sulfur. Urea composed 4% of the purified rations used.

In a later experiment, Lofgreen *et al.* (44) examined further the retention of nitrogen by lambs consuming diets of which 40% of the total nitrogen was provided by each of the following sources: urea, urea + methionine, linseed meal, and dried egg. Although only 18% as much methionine (0.2% of diet) was added to the urea-containing diet as was employed in the first experiment (48) (though 31% more urea was fed), the improved retention of nitrogen effected by methionine supplementation again was demonstrated. The biological value of the protein resulting from the basal diet supplemented with the various sources of nitrogen providing a protein equivalent of 10% were: urea, 71; urea+ methionine, 74; linseed meal, 76; and dried egg, 80. These values suggest that the nature of the dietary nitrogen influences the metabolism of nitrogen in lambs and that, in this species, the quality of protein fed may be of considerable importance. Nehring and Schramm (64) had reported earlier that the addition of cystine to urea-containing diets did not affect the utilization of urea nitrogen by lambs. The feasibility of feeding methionine to fattening lambs in the feed lot has not been determined.

Since these experiments (44, 48) were conducted, it was observed (86, 87) that lambs stored nitrogen and gained weight on purified diets to which urea contributed almost all of the nitrogen. As a result of feeding such rations to lambs and goats it was demonstrated that all 10 amino acids (including methionine) known to be essential for rat growth are synthesized in the rumen (50). The only source of sulfur in these diets was sodium sulfate. Consequently, it appeared that rumen microorganisms are able to utilize inorganic sulfur and urea nitrogen in the synthesis of methionine. Later studies (88) showed that the nitrogen of urea is not utilized when sulfur is absent from the diet. Earlier attempts (64, 66) to determine the effect of sulfur upon urea utilization appear to have failed because of the relatively high level of sulfur in the basal diets used.

Weir and Rendig (96) found that the alfalfa hays produced on two different types of soils were markedly different in sulfur content. In a feeding trial with lambs, alfalfa hay containing 0.22% of inorganic sulfur allowed a faster rate of gain than did that containing 0.08 to 0.12%. The levels of sulfate in blood serum reflected the inorganic sulfur content of the hay. In another trial, the sulfur and nitrogen contents of the two hays were equalized by the addition of methionine and urea. Lambs receiving hay and methionine gained more weight than did those receiving hay and urea, and those receiving unsupplemented hay gained at an intermediate rate. Insufficient data were provided to allow an examination of the entire rations employed in these trials. The authors (96) pointed out that the relationship of sulfur intake to growth of lambs is inconclusive.

An experiment designed to study the value of elemental sulfur for sheep fed a methionine-deficient ration has been reported (27). The three treatments imposed consisted of feeding *(a)* a basal ration low in sulfur-containing amino acids, *(b)* the basal ration + 0.5% of elemental sulfur, and *(c)* the basal ration + 0.5% of methionine. Although the sheep receiving the methionine supplement grew faster than those fed the basal diet, the addition of sulfur to the basal diet did not improve the rate of gain. The length and weight of wool produced by the sheep receiving the diets supplemented with sulfur or methionine were greater than those of sheep receiving the basal ration alone. Thomas *et al.* (88) reported a similar effect upon wool growth of sheep fed urea-containing diets supplemented with inorganic sulfates.

Klosterman *et al.* (41) reported that the addition of methionine to certain rations to which dehydrated alfalfa or field peas contributed the main portion of the nitrogen resulted in increased nitrogen retention by ewes.

The fact that various responses of sheep to methionine supplementation have been observed suggests that, with certain kinds of rations, this species may have a greater need for sulfur-containing amino acids than do other ruminants. Although Reed *et al.* (75) found the bacterial protein formed in the rumen of sheep to be deficient in methionine, no similar studies of the protein formed in the rumen of other ruminants appear to have been made. The results presented by Loosli *et al.* (50) seem to confirm the observation that ruminal protein of sheep is low in methionine. Consequently, the simplest explanation for the improved utilization of urea by lambs consuming methionine-containing diets (44, 50) is that methionine appears to supplement the bacterial protein utilized by the host animal.

Although a consistent response was not observed, an increase of approximately 10% in body weight gains of heifers in a long-time experiment conducted by Jones and Haag (37) suggested that sodium sulfate may improve the utilization of urea in certain rations. One per cent of sodium sulfate was added to the concentrates to provide 0.13% of sulfur, and the basal concentrate mixture to which 3% of urea was added contained 6.2 to 7.6% crude protein.

Satisfactory weight gains (1.4 lb. per day) were secured by Morrison *et al.* (62) in heifers fed rations containing 4.5% of urea and supplemented with 1.2% of sulfur-containing salts. These workers did not test the effects of high-urea, low-sulfur rations. As a result of supplementing the concentrate feeds for lactating cows with 1% of sodium sulfate and 0.5% of methionine singly, Jones *et al.* (38) concluded that neither of these sources of sulfur improved the efficiency of utilization of a ration low (0.1%) in sulfur.

Recently, it was demonstrated directly that sulfur in the form of sodium sulfate may be used by rumen microorganisms of the cow (11), goat (12), and sheep (11, 12) in the synthesis of cystine and methionine. After radioactive sulfur was fed as $Na_2S^{35}O_4$, S^{35} was found in the methionine and cystine isolated from either the rumen ingesta and/or the blood and milk proteins from certain of the animals.

Since sulfur and nitrogen exist in animal tissues in a ratio of approximately 1 to 15, it is indicated that the ingestion of these elements in the same ratio would ensure satisfactory performance, provided that a sufficient quantity of both is supplied and that both elements are equally well utilized. In a recent review of the sulfur needs of ruminants, Loosli (47) drew attention to the fact that of 14 common roughage feeds and 14 concentrates listed by Morrison (61) only four had a sulfur to nitrogen ratio greater than 1 to 15 or contained less sulfur than 0.1%. Of these four feeds, two possessed a ratio only slightly wider than 1 to 15. It was pointed out that the sulfur values used were determined 10 to 30 years ago and may be in error because of analytical difficulties and changing sulfur contents of feeds (47).

The results of experiments dealing with the effects of sulfur upon urea utilization suggest that only a small amount of this element is needed for the synthesis of the sulfur-containing amino acids and that when urea is used with the usual quantities of common feeds, sulfur supplementation has little, if any, value. This appears to be true particularly when rations contain more than 0.1% of sulfur. However, present information does not allow a statement of the level of sulfur in the ration below which supplementation is required. Information is needed on the amounts of sulfur required by ruminants, the availability of different forms of sulfur, and the sulfur content of feedstuffs produced in different geographic areas.

It is difficult to assess the relative biological value of protein formed from urea and that of conventional protein sources. The wide range of biological values observed in various experiments may have been effected by several quantitative and qualitative differences among the various basal diets to which the nitrogen sources were added. Such factors as the levels of nitrogen fed, the nature and amounts of nonnitrogenous substances composing the various diets, and the manner in which the experiments were conducted may account for some of the variation reported. It is clear, however, that the feeding of high levels of urea results in a low biological value of the formed protein. The results of most experiments in which urea has been fed at levels approximately maintaining nitrogen equilibrium indicate that the biological value of the protein formed is somewhat lower than the protein provided by conventional supplements.

Quality of bacterial and protozoal proteins of the rumen. The apparent similarity of the metabolic utilization of nitrogen from several sources has suggested to Johnson *et al.* (35, 36) that the protein actually available to the animal is that contained in microorganisms. The findings of Johnson *et al.* that regardless of the nitrogen source a large proportion of the nitrogen utilized by ruminant animals is in the form of protein in bacteria and that the biological value of the nitrogen of rations containing 10 to 12% crude protein is approximately 60 are the bases for this view. However, the fact that the biological value of the nitrogen of several sources provided at levels of 10 to 12% of total crude protein has been found to be as high as 71 to 81 (44, 89) would indicate that various nitrogen sources may be metabolized differently and, therefore, have

different values. In view of the wide range in the biological value of nitrogen for ruminants, it seems possible that, under certain conditions and particularly those in which certain nitrogen sources are fed, a portion of the nitrogen utilized may come directly from the dietary protein and the remainder may be provided as bacterial protein. Of course, this partition would depend upon the amount of required amino acids absorbed directly from the rumen or other parts of the digestive tract but, nevertheless, escaping bacterial action. The relatively high biological value of the proteins of various feeds fed to ruminants may be partially explained on this basis. Also, recent evidence (54, 75) demonstrates the high biological value of bacterial protein, though definite information on the amount of bacterial protein provided per unit of time to animals consuming various rations is lacking.

The quantitative studies of Duncan *et al.* (22) suggest that the amount of protein synthesized in the rumen of calves is considerable. In a study in which a ration of natural feedstuffs and a purified diet containing urea were employed, 78 and 19 to 190%, respectively, more true protein existed in the rumen 6 hours after feeding than was present prior to feeding. The increased amount of protein in the rumen of calves fed the natural diet could be accounted for by the ingested protein. Since measurements of the minimum molecular weight of the protein appeared to be characteristic of that of naturally occurring microorganisms, it was concluded that the increased concentration of protein in the rumen of urea-fed calves was due to the utilization of urea nitrogen by rumen microorganisms.

It is now well established that protein may be synthesized from nonprotein nitrogen. The synthesis of protein occurring in vitro when carbohydrates were added to the medium was found to be correlated with the increase in numbers of bacteria but was not correlated with numbers of protozoa (84). The finding that rumen protozoa appear to be unessential in the conversion of urea to protein was verified by the nitrogen-balance studies conducted by Johnosn *et al.* (36) with defaunated lambs. However, these workers observed that the number of bacteria in rumen ingesta was highest soon after feeding and that as the number of bacteria decreased after feeding, the number of protozoa increased. This suggested that protozoa were increasing in number at the expense of bacteria and that, although protozoa may not synthesize protein from urea, they may have some role in providing the host animal with protein more acceptable than that of the bacteria which they incorporate into their own bodies. Using rats, Johnson *et al.* (36) determined the digestibility and biological value of the protein of dried bacteria and protozoa. The true digestibility of the protein of a preparation of protozoa was 86, whereas that of bacteria was 55. When basal diets were supplemented with enough of each of the preparations to provide 11.6% protein, the biological value of the nitrogen provided by protozoa was 67.5 (range, 60-75) and that of the nitrogen of the bacteria was 66.3 (range, 56-73). Since it is difficult to obtain well defined separations of rumen microorganisms which are free from ingesta, the nitrogen of the preparation of bacteria employed in these studies (36) may have been

poorly digested as a result of fiber contamination. Employing a fractional centrifugation method, McNaught and her co-workers (54, 55) were able to separate what appeared to be a preparation consisting mainly of bacteria. When this preparation was fed to rats as the only source of protein at a level of 8% of the diet, the true digestibility of the nitrogen was found to be 73.2% and the biological value was 88.2 (55). Although the rumen bacteria studied by Johnson *et al.* (36) may have been poorly digested because of fiber contamination, the digestibility of the more highly refined bacterial preparation used by McNaught *et al.* (54) was only slightly higher, suggesting that protozoa may influence the digestibility of the protein by the host animal.

Others (75) have compared the value of casein with that of the protein in bacteria isolated from the rumen of sheep receiving various diets. The true digestibility of protein of bacteria taken from sheep consuming green forages was 62.1 as compared to 64.8 for bacterial protein taken from sheep receiving dry feeds. The true digestibility of casein was 101.2. When the protein sources composed 9.2 to 9.7% of the diet of growing rats the biological values were: bacterial protein from "green-fed" sheep, 79.9; bacterial protein from "dry-fed" sheep, 77.9; and casein, 79.6. The cystine contents of the two kinds of bacteria were similar, whereas the bacteria from sheep fed green forages contained more methionine than those of sheep receiving dry feeds. Based upon results obtained with growing rats, Reed *et al.* (75) concluded that bacterial protein is poorly digested, relatively high in biological value, and somewhat deficient in methionine.

Because of the large quantities needed, the digestibility and biological value of the nitrogen in preparations of bacteria and protozoa have not been studied with ruminants. No reports were found of experiments in which the biological value of protein of bacteria isolated from animals consuming urea-containing diets was determined.

USE OF UREA IN RATIONS FOR RUMINANTS

Growing cattle. That urea may be substituted for a portion of the protein in the ration of growing cattle has been demonstrated by several groups of investigators (5, 9, 25, 33, 49, 60, 62, 102).

During a 142-day feeding period, Bartlett and Cotton (9) obtained average daily gains of 0.99 lb. in heifers 7 to 17 months of age as a result of feeding a low-protein ration consisting of meadow hay, oat straw, mangels, tapioca flour, and corn. The daily addition of 0.127 lb. of urea to the basal ration yielded a daily gain of 1.23 lb. Replacement of the 1 lb. of corn in the basal ration with 1 lb. of decorticated peanut cake resulted in an average daily gain of 1.39 lb. The difference in the weight gains of heifers fed peanut cake and of those receiving urea was not significant. The difference between the gain of heifers fed the nitrogen-supplemented rations and that of heifers receiving the low-protein ration was significant.

Although only a small number of Holstein heifers were employed in their experiments, Work and Henke (102) showed that urea is utilized for growth.

Heifers receiving a concentrate mixture containing 20% of cane molasses, 20% of dried pineapple pulp, 20% of crushed oats, 10% of standard rolled barley, 8% of tuna fish meal, 20% of soybean oil meal, 1% each of salt and steamed bone meal, and vitamin supplements gained 1.4 lb. per day during the 52-week feeding period. Heifers receiving cane molasses, dried beet pulp, crushd oats, and rolled barley in the proportion of 20:30:21:23, supplemented with 4% of urea and the same mineral and vitamin supplements, gained 1.15 lb. per day. The feeding of a mixture of the four basal ingredients in the proportion of 20:24:24:30 without nitrogen supplementation resulted in a daily gain of 1.0 lb. per day.

The protein-equivalent contents of the three concentrates were 20.57, 20.69, and 8.40%, respectively. Fresh Napier grass was fed to all heifers. Under the conditions of this experiment it was demonstrated that the feeding of conventional nitrogenous feeds results in greater growth of heifers than does feeding of urea. A greater difference between the growth response of heifers consuming the low-protein concentrate and those receiving the urea-supplemented concentrate might have resulted had the basal ration contained less of crude protein. No abnormal physiological effects, such as diuresis, resulted from the feeding of urea.

During two winters, the feeding of pellets containing 4% of urea, 75% of cottonseed meal, 11% of hominy feed, and 10% of molasses produced the same weight gains in Hereford heifers grazing dry winter grass as did the feeding of cottonseed meal (14). Approximately 2.3 lb. of these supplements were fed daily. The average daily weight gains ranged from 0.26 to 0.33 lb. Average initial weights of the heifers were 700 lb. for the first year and 638 lb. for the second year.

Baker (5) studied the response of beef heifer calves (average initial weight, 432 lb.) to various protein supplements fed during a 112-day period. Corn silage, corn grain, and mineral supplements composed the basal diet. In addition to 29.17 lb. of corn silage, the four rations consisted of: 1.97 lb. corn + 0.99 lb. soybean oil meal; 1.65 lb. corn + 1.32 lb. wheat distillers' dried grains with solubles; 0.99 lb. corn + 1.97 lb. wheat distillers' dried grains with solubles, and 2.85 lb. corn and 0.11 lb. urea. The average daily weight gains of the calves of the respective lots were 1.53, 1.32, 1.33, and 1.27 lb. Although the response to urea was inferior to that produced by soybean meal, these data indicate that the protein replacement value of the nitrogen of urea is approximately equivalent to that of wheat distillers' dried grains for wintering beef calves.

In another experiment (7), urea, wheat distillers' dried grains, and ground rye were compared singly with soybean oil meal for wintering beef steer calves during a 140-day feeding period. The calves had an average weight of 427 lb. at the beginning of the period. Mineral supplements and a full feed of sorghum silage were provided. The following amounts of supplements were fed to the respective lots (per animal daily): 1.0 lb. corn; 1.0 lb. mixture of corn and urea (providing the same amount of nitrogen as that supplied by 1.0 lb. of

soybean oil meal); 1.0 lb. soybean oil meal; 1.29 lb. wheat distillers' dried grains; 2.0 lb. wheat distillers' dried grains; and 2.0 lb. ground rye. The average daily weight gains were: 0.81, 1.07, 1.39, 1.36, 1.50, and 1.22 lb., respectively. These data indicated that the nitrogen of urea was not utilized as well as that of soybean meal or wheat distillers' dried grains; however, greater gains resulted from the feeding of urea with corn than from the feeding of corn alone as a supplement to the silage. Eleven pounds of urea reduced the amount of feed required per 100 lb. of gain by 41 lb. of corn and 550 lb. of silage.

Satisfactory results were obtained with beef heifer calves wintered on dry grass supplemented with urea-cottonseed meal pellets in which 25% of the total nitrogen was provided by urea (15). An average daily gain of 0.34 lb. was obtained on this diet, whereas supplementation of the same grass with cottonseed meal produced gains of 0.46 lb.

Studies of the relative value of cottonseed cake, soybean cake, and urea-cottonseed meal pellets for 2- and 3-year-old steers grazing dry bluestem grasses have been reported by Ross *et al.* (77). These experiments were conducted during three successive winter periods having an average duration of 144 days. Thirty Hereford steers composed each treatment lot. The weight gain of animals receiving the soybean cake was much greater than that of the steers fed either cottonseed cake or the urea-cottonseed pellets. Since the "urea-cottonseed meal" pellets contained 75% of cottonseed meal (as well as 4% of urea, 11% of hominy feed, and 10% of molasses), the authors (77) stated that the nitrogen needs of the steers may have been satisfied by the cottonseed meal alone.

As a result of feeding a basal ration consisting of 30% yellow corn, 20% starch, 47% timothy hay, and supplementary minerals and vitamins and containing slightly less than 6% crude protein, Wisconsin workers (33) found that a male calf gained approximately 120 lb. during a 28-week period. When urea and casein were added singly to the same basal ration in quantities sufficient to provide rations containing 18 per cent of protein equivalent, the weight gains by animals receiving these two rations during the same period of time were approximately 220 and 310 lb., respectively. Although the age of these animals at the beginning of the trial was not stated, it is assumed that the calves were 4 to 5 months old and weighed approximately 300 lb., since they were consuming approximately 8 lb. of dry matter daily. During a 14-week period immediately following the first 28-week feeding period, molasses was added to the rations to stimulate appetite. The weight gains recorded over the 14-week period in which molasses was fed were: low-protein basal ration, 65 lb.; low-protein basal ration + urea, 110 lb.; and low-protein basal ration + casein, 126 lb. The protein equivalent of the rations containing urea or casein was 18%.

In an experiment with female calves weighing 250 to 290 lb., Wisconsin workers (33) employed a low-protein (5.38%) basal ration of approximately 19% of corn, 23% of starch, 10% of corn molasses, 45% of timothy hay, and

supplementary minerals and vitamins. Urea was added to the basal ration in sufficient quantities (1.4, 2.8, and 4.3 lb. of urea, respectively, per 100 lb. of total ration) to compound rations containing 9.49, 13.70, and 17.64% of protein equivalent. A ration (15.52% crude protein) of which 11% of the dry matter was composed of casein was tested also. The weight gains during a 12-week feeding period begun when the heifers were approximately 4-5 months old were: basal (5.38% of protein equivalent), 0 lb.; basal + urea (9.49% of protein equivalent), 70 lb.; basal + urea (17.64% of protein equivalent), 52 lb.; and basal + casein (15.52% of total protein), 74 lb. The basal ration was changed by the addition of 1.4% of urea (making a protein equivalent of 9.49%) when the animal receiving this ration was 12 weeks of age. This animal then gained in weight at a rate of 1 lb. per day. The feeding of these rations was continued over a 12-month period, at the end of which all heifers were slaughtered for carcass analyses. The animals receiving the lowest level (1.4%) of urea and the animal receiving the casein-supplemented ration grew at a rate of more than 1 lb. per day during the 12-month feeding period. The animals receiving the rations supplemented with the higher levels (2.8 and 4.3%) of urea did not gain as rapidly and averaged about 0.9 lb. per day. Because of the small number of animals used in these experiments, the observations relative to level of urea fed should not be considered as final.

Examinations of the blood of these animals showed that the level of protein was within the normal range and that, with the exception of the animal receiving the high-urea ration, there was no increase in the level of urea (33). The composition of the carcasses of the urea-fed animals was similar to that of the animal receiving the casein-supplemented ration, suggesting that the increments in body weight were of normal protein content.

Morrison *et al.* (62) recently demonstrated satisfactory growth in dairy calves fed high-urea rations. A ration consisting of wheat straw, ground snap corn, cane molasses, minerals, vitamins, and 4.5% of urea was compared with a similar ration in which 25.5% of cottonseed meal replaced the urea. During a 97-day period, calves fed the urea-containing ration gained 1.0 lb. of body weight daily, whereas those consuming the cottonseed meal-containing ration gained 1.4 lb. per day. Although the difference in weight gains was marked, it appeared that the difference in nutrient intakes, rather than the difference in source of nitrogen, was responsible for these results. More of the ration containing cottonseed meal was consumed, and this ration provided approximately 10% more net energy (estimated) than did the urea-containing ration. During the subsequent 91-day period, the urea-containing ration was altered by substituting ground corn for the ground snap corn. This nearly equalized the estimated net energy value of the two rations. During the second period the weight gains (1.4 lb. per day) of calves fed urea were about the same as those (1.6 lb. per day) fed cottonseed meal as the main source of nitrogen. The urea-containing rations employed in these studies provided approximately twice as much sulfur (0.21 to 0.35%) as did the rations containing cottonseed meal. Since the amount of nitrogen in the rations was reduced as the calves

became larger, the urea-containing rations contained from 16.3 to 13.1% protein equivalent, of which urea provided about 70%.

The animals used in experiments (9, 33, 62) previously described appear to have been at least 4 months old when the feeding of urea was begun; therefore, the rumina of these animals probably were well developed microbiologically. An experiment (49) conducted at Cornell in which 2-month old calves were fed urea-supplemented rations showed that, even at this early age, calves are able to utilize urea to a considerable extent. In this test, the basal ration containing 4.4% of conventional protein was supplemented with 4% of urea to form a ration containing 11.8% of protein equivalent. The calves receiving the basal diet grew at a rate much slower than normal, whereas those receiving the urea-supplemented diet grew at a fairly satisfactory rate, although somewhat slower than is considered normal. The nitrogen balance of the calves receiving the basal ration was negative, whereas that of calves receiving the urea-supplemented diet was positive. The calves receiving the diet containing urea retained 24 to 36% of the dietary nitrogen. Digestibility of the dry matter and carbohydrates was markedly improved by the addition of urea to the basal diet. As a result of carcass analyses, it was found that the nitrogen content of the body was about normal, though the weight gained consisted of a higher proportion of water and less fat than is considered to be normal (49).

From the studies of cattle growth it may be concluded that urea feeding is advantageous when the true protein content of the ration is low and when the quantity of available carbohydrates is high. Although 2-month old calves are able to utilize some urea, it does not seem advisable to feed it to calves less than 4 months of age. The findings reviewed here indicate that the nitrogen of urea is somewhat inferior to that of conventional protein supplements for growing cattle, although the composition of the remainder of the diet may modify the difference in response.

Fattening cattle. In a study conducted by Briggs *et al.* (14), daily weight gains of 1.62 lb. were secured with yearling cattle (weighing 540 lb. initially) fed a urea-containing ration, whereas similar animals gained 2.03 lb. while consuming a basal diet supplemented with cottonseed meal. The basal diet consisted of corn, wheat, oats, and prairie hay; 0.16 lb. of molasses was added daily to the urea-containing diet. Some of the difference in the weight gains obtained in this experiment (14) seems to be accounted for by the lower intake of nutrients by the steers receiving the urea-containing diet. The authors drew attention to the failure of the steers to consume the entire ration allowed, presumably because of the unpalatability of urea. The basal diet employed in this test appears to have contained too much protein (approximately 10.6%) to allow the value of urea as a protein replacement to be determined.

Briggs *et al.* conducted further tests in which different levels of urea and cottonseed meal were compared for fattening calves. During one year, the basal ration consisted of approximately 76% corn and 13% prairie hay. A control diet was prepared by the addition of approximately 11.5% of cotton-

seed meal. The value of this diet for fattening was compared to two urea-containing rations, one consisting of about 8.2% of cottonseed meal, 0.44% of urea, and about 1.1% each of molasses and hominy feed, and the other consisting of about 5.3% of cottonseed meal, 0.86% of urea, 1% of molasses and 3.4% of hominy feed. Each of the three rations contained approximately 12.3 per cent protein equivalent. The average daily weight gains were: control, 1.81 lb.; 0.44% urea ration, 1.95 lb.; and 0.86% urea ration, 1.98 lb. Although these data indicated that slightly greater gains were obtained as a result of feeding urea, it would be of interest to know what the gains would have been had no urea been added. If it is assumed that the digestion coefficient for the crude protein of the various components of the basal diets were: cottonseed meal, 58; prairie hay, 37; corn, 76; hominy feed, 71; and molasses, 0; then the amounts of digestible crude protein provided daily by the basal diets (urea free) were approximately 1.2 and 1.1 lb. per animal. For animals of the size (572 to 589 lb., average) employed in these experiments, the lower level of digestible protein suggested by Morrison (61) is approximately 1.2 lb. daily.

During a second year similar tests were made. The basal diet consisted of approximately 73% corn and 13% prairie hay. The control diet was compounded by adding 14% of cottonseed meal. Two experimental diets were formed by adding to the basal diet: *(a)* 10.63% of cottonseed meal, 0.57% of urea, 1.4% of molasses, and 1.6% of hominy feed, and *(b)* 6.2, 0.99, 1.2, and 4.0% of these constituents, respectively. The average daily weight gains secured as a result of feeding these diets to calves were: control, 1.85 lb.; 0.57% urea diet, 1.77 lb.; and 0.99% urea diet, 1.61 lb. The three diets provided the following percentages of protein equivalent: control, 13.5; 0.57% urea diet, 13.4; and 0.99% urea diet, 12.9. Weight gains of the calves closely paralleled the rate of feed consumption. The authors pointed out that near the end of the 167-day feeding period the animals receiving the higher level of urea (0.99% of the diet) refused to consume the entire allowance of urea pellets. Since, in this test, the amount of digestible protein provided daily by the basal diets (urea-free, but containing cottonseed meal, hominy feed, and molasses) was estimated to be 1.1 and 0.9 lb. per animal, the value of urea for fattening was subjected to a more critical examination during the second year than during the first year. A more satisfactory study could have been made had a negative-control treatment been employed in these experiments.

In these studies (14) the nitrogen of urea composed from 9 to 20% of the total dietary nitrogen. Since the animals receiving the highest level (0.99%) of urea refused to consume their total allowance of urea pellets (also containing cottonseed meal, hominy feed, and molasses), Briggs *et al.* cautioned that, when urea is fed in pelleted form at this level, unsatisfactory results may be expected in long-time feeding operations.

In other experiments conducted by the Oklahoma workers (78), the value of urea as a partial substitute for cottonseed meal in the ration of fattening beef calves was studied. A basal diet of ground corn, sorghum silage, and alfalfa hay was used. The addition of cottonseed meal or urea-cottonseed meal

pellets to the basal diet effected daily gains of 2.07 to 2.13 lb. per steer. Cottonseed meal contributed 75 or 50% of the nitrogen provided by the urea-cottonseed meal pellets. Consequently, since the diets to which urea was added appear to have provided almost enough nitrogen for animals of the size used, it is difficult to assess the value of urea in this test. These trials were continued (79) during a subsequent year. The average daily weight gains ranged from 2.14 to 2.22 lb. per steer, with the urea-fed animals having a slight advantage. As in the previous test, no negative control was employed. Since the two basal diets to which urea was added contained approximately 10 and 12% crude protein, no actual benefits may have been derived from urea.

In more recent experiments at the Oklahoma station (46), the basal diets (containing corn, alfalfa hay, and sorghum silage) provided approximately 8.9 to 9.7% crude protein. When enough cottonseed meal or urea-cottonseed meal pellets was added to the basal diet to provide approximately 10.6% protein equivalent, steer calves gained at a rate of about 1.8 lb. per head daily. Increasing the amount of cottonseed meal in urea-free diets to provide levels of 11.4 and 12.4% crude protein resulted in average daily gains of 1.98 and 2.18 lb., respectively. Whether urea provided in sufficient amounts to form a diet of 11 to 12% of protein equivalent would have given results similar to those obtained with cottonseed meal was not examined in this experiment. However, this test provided further evidence for the value of urea as a protein replacement in the diet of fattening beef calves.

These results were confirmed during a subsequent year (45). The addition of sufficient cottonseed meal to corn-alfalfa hay-sorghum silage rations (8.9 to 9.5% crude protein) to provide approximately 13.1, 12.1 and 11.0% crude protein resulted in daily average gains of 2.22, 2.06, and 1.95 lb., respectively. Urea added to a similar basal diet (9.7% crude protein) to increase the amount of protein equivalent to approximately 12.5% resulted in an average gain of 1.8 lb. per steer daily. The steers fed the urea-containing diet consumed about 10% less corn daily than the steers fed cottonseed meal. Although the authors stated that the urea pellets were slightly unpalatable, it seems possible that the feeding of urea at this level may have yielded gains similar to those obtained with cottonseed meal in the 12% crude protein diet if an equivalent amount of nutrients had been consumed. If the unpalatability of urea was the primary cause of the low intake of nutrients in these experiments, it appears that a level of urea of 0.97% of the total diet is not satisfactory for fattening calves. A similar observation was reported earlier by the Oklahoma workers (14).

Baker (6) found urea to be an effective substitute for soybean oil meal for fattening yearling steers weighing approximately 630 lb. initially and fed during a 159-day period. A basal ration containing approximately 21 lb. of corn silage and 17.2 lb. of corn was fed. When soybean oil meal was employed, it replaced an equal quantity of corn in the basal ration, resulting in an average daily intake of 17.2 lb. of concentrates. The following amounts of nitrogenous feeds were either substituted for corn or (in the case of urea) added to the

basal ration to form the experimental rations: 1 lb. soybean oil meal; 0.114 lb. urea; 1.5 lb. soybean oil meal; 1 lb. soybean oil meal + 0.0575 lb. urea; 0.5 lb. soybean oil meal + 0.114 lb. urea, and 0.172 lb. urea. The average weight gained per steer daily ranged from 2.60 to 2.71 lb. for the six treatments. Since, according to the Morrison feeding standards (61), the minimum allowance of digestible protein for steers of the size employed in these studies is 1.52 lb. per steer daily and since it was estimated that the basal ration employed provided 1.37 lb. of digestible protein, a good test of the value of the nitrogenous supplements used was effected. Of the total nitrogen in the two urea-containing rations free of soybean oil meal, urea contributed about 18 and 25%, respectively. The results of these trials indicated that urea and soybean meal were approximately equivalent for fattening yearling steers when the dry matter of the total diet provided 10 to 11% protein equivalent.

A study of the relative value of urea, wheat bran, and cottonseed meal for fattening yearling steers has been reported (21). The supplementation of a diet containing prairie hay and ground shelled corn with pellets containing cottonseed meal, bran, and urea resulted in an average daily weight gain of 2.03 lb., whereas the same amount of cottonseed meal alone yielded a gain of 2.20 lb. per day. Pelleted mixtures of urea and bran and cottonseed meal and bran as supplements to the corn-prairie hay diet resulted in average daily gains of 1.93 and 2.03 lb., respectively. Steers receiving only corn and prairie hay gained 1.74 lb. per day. These results suggest that the nitrogen of urea was utilized to about the same extent as that of bran but was not as efficiently used as that of cottonseed meal. Less feed was required per unit of gain by the steers receiving cottonseed meal than those fed the other supplements. Steers fed the urea-supplemented diet required more feed per unit of gain than the steers of any other lot except the negative controls.

Kirk (40) examined the protein replacement value of urea for cottonseed meal when fed with citrus feeds to fattening beef cattle. A basal ration of hay, citrus pulp, and citrus molasses was employed. When cottonseed meal was used as a supplement to the basal ration, the average daily gains of calves and yearlings were 2.04 and 2.53 lb., respectively. The substitution of urea and citrus pulp for 40% of the cottonseed meal resulted in average gains of 1.81 and 2.45 lb. per day in calves and yearlings, respectively. Urea was not as effective for fattening calves as for fattening yearlings. About the same amount of feed was required per unit of gain for yearlings receiving urea as for those fed cottonseed meal as the main source of nitrogen. However, the urea-fed calves required more feed per unit of gain than the calves fed cottonseed meal.

In another experiment, Kirk (40) fed to yearlings grazing native pasture *(a)* citrus molasses and cottonseed meal and *(b)* citrus molasses containing 3% of urea and citrus meal. The amounts of nitrogen and of digestible nutrients in the two rations were approximately the same. The animals receiving the cottonseed meal-containing supplement gained an average of 1.68 lb. per day as compared to 1.59 lb. for those fed the urea-containing supplement.

In what appears to be one of the most carefully conducted investigations of the value of urea for fattening cattle, Watson *et al.* (91) used a combination feeding trial, metabolism, and slaughter experiment. Thirty beef calves weighing 330 to 440 lb. initially were fed experimentally during periods of 280 to 350 days. Three treatments were imposed. The negative control group received a basal ration consisting of timothy hay, oat straw, barley, corn starch, corn oil, and molasses and providing approximately 4.3% crude protein. The second treatment consisted of feeding enough casein in addition to the basal ration to satisfy the Morrison allowances (61) when starch and oil were reduced to equalize the digestible nutrient content. In a third treatment the basal ration was supplemented with enough urea to provide the same amount of digestible nitrogen furnished by casein in the second treatment. All rations were fed in amounts sufficient to provide digestible nutrients at levels allowed by the Morrison standards. The animals composing the negative-control group gained very little weight, whereas those receiving the casein- and urea-supplemented rations gained 887 and 619%, respectively, more weight than the negative-control calves. Urea-fed calves gained 70% as much weight as those fed casein.

Since some representative calves were slaughtered prior and some subsequent to the feeding trial, Watson *et al.* were able to ascertain the proximate composition of the weight gained. Although crude protein was lost from the bodies of negative control animals, urea- and casein-fed animals gained considerable protein. A slightly higher proportion of the gain consisted of protein in casein-fed calves (18.6%) than in urea-fed calves (17.7%). Also, the gains of the calves fed casein consisted of a higher proportion of mineral matter than those of calves fed urea. Mineral matter was lost from the bodies of negative controls. More than 80% of the weight gained by negative control calves consisted of fat; the remainder was water. Fat constituted a greater proportion of the gain of calves fed urea (25.5%) than it did in calves fed casein (19.7%).

Aside from the effects of the two sources of nitrogen employed in these studies upon the amount of weight gained, it is interesting to note that the composition of the substance gained by animals receiving similar rations (except for source of nitrogen) may be so different. Although 70% as much weight was gained by calves receiving urea as by those fed casein, the magnitude of this difference was not nearly so great calorie-wise. The gain in energy by urea-fed calves was approximately 81% as great as that made by casein-fed calves.

Probably some of the variation in the response of fattening cattle to the feeding of urea is associated with the nature of the different rations employed. The results of some of the experiments reviewed are difficult to analyze because of a lack of negative controls, the addition of urea to basal rations which, alone, provided practically enough nitrogen to satisfy the needs, and, in some cases, because of a lower nutrient intake by animals receiving the urea-containing rations than by those receiving the positive control rations. The results of

other experiments indicate that conventional nitrogenous feeds are only slightly superior to urea for fattening cattle when urea replaces up to 25% of the nitrogen of a total ration containing 11 to 13% of protein equivalent. When urea was substituted for 40 to 60% of the nitrogen of the total ration, the superiority of common high-protein feeds over urea seemed to be more pronounced (91). The difference in the responses to urea and conventional protein feeds generally has been more marked in calves than in yearlings or older cattle.

Growing, fattening lambs. Results of metabolic experiments demonstrate that growing lambs are able to utilize at least some urea (29, 30, 35, 44, 48). Some of the results obtained [particularly those of early experiments (51, 64, 65, 100)] are variable. Although it is difficult to account for the variable protein-replacement value of urea found in the various experiments, the composition of the nonnitrogenous portion of the diets used has been a major difference.

Mangold (51) found that urea satisfactorily replaced most of the nitrogen provided by linseed oil meal to a ration of beet pulp, molasses, and bran fed to lambs. He concluded that urea nitrogen was almost completely utilized. The addition of urea to the low-protein basal diet effected an improved digestibility of nitrogen. Others (64, 65) have found that the body-weight gains of lambs fed urea-supplemented rations were similar to those of lambs fed conventional protein feeds. In one experiment the basal rations employed during a 14-month feeding period consisted of straw, molasses, starch, and small quantities of purified proteins (65).

In balance experiments, Miller and Morrison (56) found the following percentages of dietary nitrogen to be stored by lambs consuming semi-purified rations in which the various supplements provided almost all of the nitrogen: linseed meal, 22.7; nonfat dry milk solids, 19.8; casein, 20.4; and urea, 2.0. Urea was utilized, but to an extent little more than enough to maintain nitrogen equilibrium. A poor utilization of urea nitrogen by lambs was reported also by Wohlbier and Windheuser (100). As a result of conducting nitrogen-balance experiments, these workers concluded that very little, if any, urea nitrogen is deposited as protein in the animal body.

Johnson *et al.* (35) found that the addition of urea to a low-protein diet to provide 12% protein equivalent effected a retention of nitrogen which would not be improved by further additions of urea. However, increasing the true protein content (with conventional protein sources) improved nitrogen retention. These studies showed further that the protein of soybean meal or of casein was digested and retained to about the same extent as that formed from urea when each was fed at levels of 11 to 12% protein equivalent. In their experiments, Johnson *et al.* found that rations containing 16 to 17% protein equivalent of which 65 or 46% was provided by urea would not satisfy the nitrogen requirements of growing lambs. The basal rations contained 0.1% of sodium sulfate. Harris and Mitchell (31) had reported previously that the nitrogen of casein is superior to that of urea for growing lambs.

The results of more recent experiments conducted by Hamilton *et al.* (29) largely confirm those (35) reported earlier. When the protein equivalent of rations did not exceed 12% and when at least 16% of the total nitrogen was provided by conventional feeds, the nitrogen of urea was found to be as well utilized by growing lambs as the same amount of nitrogen supplied by nonfat dry milk solids, nonfat dry milk solids + cystine, corn gluten feed, casein, or casein + cystine. The nitrogen of linseed oil meal, however, was more efficiently utilized than that of urea when fed in rations containing about 12% protein equivalent. Hamilton *et al.* found that the nitrogen of a ration providing 16.2% protein equivalent of which urea supplied 63% was not as efficiently utilized as that of a ration containing 11.4% protein of which urea provided 46%. These workers concluded that urea is as satisfactory a source of nitrogen for growing lambs as most ordinary feeds, provided that the protein equivalent of the ration does not exceed 12% and that at least 25% of the dietary nitrogen is supplied by conventional protein sources.

Recently, as a result of supplementing a low-protein (7.07%) ration for lambs with enough urea to provide rations having protein-equivalent contents of 8.45, 10.13, and 12.32%, Gallup *et al.* (26) found that maximum retention of nitrogen is obtained at a level of protein equivalent of about 10%. A slightly higher retention was attained when cottonseed meal was employed as a supplement to furnish 12% crude protein.

In feed-lot trials, Willman *et al.* (99) found that the weight gains of lambs receiving a ration of shelled corn, corn silage, and mixed hay, supplemented with urea, were somewhat below those considered to be economical. A comparison of urea with linseed meal was made in trials conducted during four consecutive years. A total of 84 lambs composed each treatment group. The urea-containing ration provided 12.3% protein equivalent and the linseed-meal ration furnished 11.3% crude protein. Average daily gains of 0.26 and 0.32 lb., respectively, were obtained. Lambs consuming rations in which all of the linseed meal was replaced by urea showed poor appetites. The addition of cane molasses and sodium sulfate did not appear to improve the utilization of urea. During these studies no attempt was made to determine the amount of linseed meal which could be replaced by urea.

The amount of nitrogen stored by lambs consuming prairie hay supplemented with urea-cottonseed meal pellets in which urea nitrogen replaced 25% of the nitrogen of cottonseed meal was about the same as that stored by lambs receiving the same basal ration and cottonseed meal (16). These rations furnished 12.6 to 13.1% protein equivalent. Replacement of 50 or 75% of the nitrogen of cottonseed meal with urea resulted in a decreased storage of nitrogen.

In feed-lot trials (71), the feeding of a basal ration consisting of corn, cottonseed meal, dried beet pulp, and prairie hay and containing 8.9% crude protein yielded the same average daily weight gain (0.37 lb. per lamb) as that obtained with the same basal ration supplemented with enough urea to provide a ration of 11.6% protein equivalent. Increasing the amount of cotton-

seed meal and decreasing the amount of urea to provide 11.5% protein equivalent also resulted in a gain of 0.37 lb. of body weight. When no urea was fed and the amount of cottonseed meal was increased to form a ration furnishing 11.0% crude protein, lambs gained 0.4 lb. per head daily. Although all four treatments resulted in satisfactory weight gains and financial returns, no apparent beneficial effects were derived from the additional nitrogen provided by urea to the cottonseed meal-containing rations used in these tests. The additional gain obtained in lambs fed cottonseed meal in the 11% crude protein ration may have been the result of a higher nutrient intake rather than of the source of nitrogen fed.

The effectiveness of urea as a protein replacement for growing fattening lambs is not as clear cut as it is for cattle. In the early experiments the high-protein levels of the basal diets used tended to yield confusing results. More recent trials in which urea was substituted for all of the protein supplement have not been very successful, and in only limited experimentation has the value of urea for a part of the protein supplement been examined. There is some evidence suggesting that urea may be used satisfactorily when a ration contains no more than 12% protein equivalent of which at least one-fourth of the nitrogen is supplied by conventional protein feeds. Other data indicate that the replacement of more than 25% of the protein supplement with urea gives unsatisfactory results. The addition of urea to rations containing 12% or more of protein equivalent has not been successful.

Lactating cows. Prior to the second World War, variable success was had in experiments dealing with the use of urea in rations of milking cows. During a 76-day period the milk production of five cows fed a ration containing conventional nitrogen sources declined 17.2 lb., and the production of the same number of cows fed a urea-containing ration declined 20 lb. (83). The average milk yield of the normally fed cows was 49.5 lb. per day and that of the urea-fed cows was 48.6 lb. The urea-fed cows lost an average of 8.8 lb., whereas the normally fed animals gained 11 lb. during the experimental period. A negative-control group was not employed in these studies.

In similarly planned tests, Ehrenberg *et al.* (23, 24) confirmed the protein-sparing influence of urea. A comparison of linseed oil cake and urea made during a 10-week feeding period indicated that urea nitrogen had a value for milk production of about 40% of that of the oil cake. In this test, urea was substituted for all of the linseed oil cake. Body weight was maintained best by cows fed urea, fairly well by those fed linseed oil cake, and least well by cows receiving the low-protein basal ration. These workers (23) concluded that, when urea replaced the protein required for the production of 15 to 18 lb. of milk produced by a cow yielding a total of 37 lb., less than 50% of the urea nitrogen was utilized. The utilization of urea was believed to exceed 50% when it replaced only the amount of protein required for the production of 9 to 11 lb. of milk. Nehring (63) also drew attention to the extent to which urea may replace protein in the rations of milking cows. His data indicated that urea could replace the protein needed for the production of 11 lb. of milk

but when it was employed to replace that required for a yield of 22 lb., milk production declined.

Richter and Herbst (76) conducted an experiment in which each of four treatments was tested with eight cows during a period of 14 weeks. The concentrate portion of the ration was designated as follows: *(a)* low-protein concentrate mixture providing 66% as much nitrogen as the "normal-protein" mixture, *(b)* "normal-protein" mixture, *(c)* concentrate mixture of which 50% of the nitrogen was provided by urea, and *(d)* concentrate mixture of which 50% of the nitrogen was supplied by glycine. All treatments resulted in a reduced milk yield and a loss of body weight. The reduction in milk production was less for cows receiving glycine than for those fed urea. Nine per cent less milk was produced by cows fed urea than by those fed the normal-protein ration. Weight was maintained best by cows fed glycine, next best by those fed the normal protein-containing concentrates, and poorest by cows fed urea and the low-protein ration. Considerable amounts of weight were lost, however, by all cows regardless of the source of the nitrogen fed.

In other experiments, only slight reductions in milk and fat yields resulted from feeding rations in which urea replaced 50% of the nitrogen (81, 82). The control animals fed a ration of conventional feeds were identical-twin mates of the urea-fed cows. These workers concluded that unless the nitrogen allowance for maintenance set forth in accepted standards is incorrect and/or that the nitrogen of a protein-deficient ration is utilized more efficiently than that of a protein-adequate ration, urea has a protein-sparing effect.

Owen *et al.* (67) compared the production of milk by cows receiving urea with that of cows fed blood meal. A low-protein basal ration and the same basal ration supplemented with blood meal or urea were fed alternately during periods of 12 to 44 days. Urea was substituted for all of the nitrogen (25% of nitrogen of total ration) provided by blood meal and a sufficient amount of starch was added to the urea-containing ration to furnish the same amount of energy as was supplied by the ration containing blood meal. The yield of milk by five of the seven cows was maintained well when urea replaced blood meal. The removal of urea from the rations of five cows resulted in a rapid decrease in the milk yield of four of the cows. One cow did not seem to be benefited by the inclusion of urea, and the data obtained with another cow were difficult to interpret.

Body weight was lost during the periods when the basal ration was fed. Although no great changes in body weight occurred when either urea or blood meal was fed, weight was maintained slightly more satisfactorily during the urea-feeding periods than during periods when blood meal was fed.

As a result of determining nitrogen balances, it was concluded that about 25% (range 12-47%) of the ingested urea was not utilized. The apparent wastage of urea nitrogen was reduced when a period during which the low-protein ration was fed preceded the urea-feeding period. These workers found that the feeding of urea had no effect upon the percentage of protein, fat, lactose, or total solids in the milk.

During and after the second World War the results of a number of experiments provided further evidence that urea may be employed as a substitute for a part of the protein in the rations of milking cows. In a study made by Rupel, *et al.* (80) during three successive lactation periods, a basal ration consisting of timothy hay, corn silage, and a concentrate mixture (10% crude protein) of approximately equal parts of corn and oats and containing minerals and vitamins was compared to the same ration supplemented singly with linseed meal and urea in sufficient quantity to provide concentrate feeds containing 18% protein equivalent. Each of 15 cows was fed each of the three rations during the three lactation periods involved, though one ration was fed continuously during a given lactation period.

The average amounts of milk (adjusted to a fat content of 4%) produced per cow per lactation period were: basal ration, 6,675 lb.; basal ration + linseed meal, 7,790 lb.; and basal ration + urea, 7,690 lb. Since the ration consumed by the cows receiving linseed meal provided approximately 0.16 lb. more digestible nutrients daily than that furnished by the urea-containing ration, it appears that some of the difference in yield on the two experimental rations may be explained on this basis. Assuming that 0.32 lb. of digestible nutrients are required for the daily production of 1 lb. of milk, the urea-fed cows might have produced 7,822 lb. per lactation period, if their nutrient intake had been equivalent to that of cows fed the linseed meal-containing ration.

Since the milk yield of cows increases from the time of the first lactation to that of maturity, Rupel *et al.* adjusted their milk-production data for age of the cows employed. On this basis, the milk yields (corrected to 4% fat content) per cow per lactation period were: basal ration, 7,855 lb.; basal ration + linseed meal, 9,355 lb.; and basal ration + urea, 8,988 lb. If the nutrient intake of the urea-fed cows is adjusted to that of the cows fed linseed meal, the milk yield (corrected for age) of the urea-fed cows becomes approximately 9,142 lb. Though the milk yield of cows receiving urea or linseed meal was greater than that of cows fed the low-protein basal ration, there was no significant difference between the yields of cows fed the two nitrogenous supplements.

Cows receiving the urea-containing ration maintained body weight about as well as those fed rations supplemented with linseed meal and considerably better than the cows receiving the basal ration. The treatments imposed in these experiments did not result in differences in the composition of the milk or blood, the flavor of milk, birth weight of calves, or the ease with which conception occurred.

These results demonstrated that urea composing as much as 1% of the total ration (or as much as 3% of the concentrate mixture) is an effective substitute for linseed meal. Urea contributed 46% of the nitrogen of the concentrate feed and 27% of the nitrogen of the total ration fed in this test.

In a series of continuous-feeding and double-reversal experiments conducted by Archibald (1), urea was compared with a combination of cottonseed meal, soybean meal, and corn gluten feed. These tests involved two successive

lactation periods. The average daily milk yields determined in the double-reversal trials during two lactation periods were: control ration, 41.4 and 43.2 lb. and urea-containing ration, 39.7 and 41.1 lb., respectively. Although Archibald pointed out that during the early stages of the experiment the control concentrate mixture was more readily accepted than the urea-containing concentrate, it seems unlikely that unpalatability is the cause of the low intake of concentrates containing urea as compared to that of the concentrates containing cottonseed meal, soybean meal, and corn gluten feed. Assuming that the intakes of mixed-grass hay, corn silage, and beet pulp were the same for both groups of cows, the differences (though insignificant) in milk yields obtained in the double-reversal trials could be practically accounted for by the differences in amounts of concentrates consumed.

Archibald pointed out that the shrinkage in milk flow was a little less when the urea ration was replaced by the control ration than when the urea ration replaced the control ration. The average daily yields of eight cows fed continuously during two successive lactation periods were: control ration, 37.5 and 42.2 lb., respectively, and urea-containing ration, 38.8 and 40.4 lb., respectively.

Although the average gain in weight favored the control ration, the difference was not significant. The weight gains of cows from just after calving time to the time immediately preceding the next calving during each of two years were: control ration, 239 and 274 lb., and urea-containing ration, 229 and 266 lb., respectively. Estimates of general condition based upon visual assessment of texture of hair and hide and degree of fleshing favored the control ration.

The length of the lactation period, reproductive performance, amounts of urea in blood and milk, and the flavor of milk were not different for the two treatments imposed.

It should be pointed out that the control concentrate mixture contained 19.9% crude protein, and the urea-containing mixture contained 17.6% protein equivalent. In view of the fact that a fair grade non-legume hay, corn silage, and beet pulp composed the roughage portion of the ration, the very small differences in the average data favoring the control ration might not have existed had the urea-containing concentrate provided as much protein equivalent as the control mixture. Urea contributed approximately 42 and 25% of the total nitrogen to the concentrate portion of the ration and the total ration, respectively. The success experienced by Archibald in feeding urea is of further interest because the production level of the cows used in his tests was much greater than that of the cows employed by Rupel *et al.* (80).

Willett *et al.* (98) conducted trials in Hawaii in which urea was added to a ration containing cane molasses, pineapple bran, corn or barley, soybean oil meal, and Napier grass to increase the protein equivalent of the concentrate portion of the ration from 7.4 to 13.2%. The urea-containing ration was compared in a double-reversal trial with a ration containing 13.4% crude protein and prepared by the addition of soybean oil meal to the basal ration. Six cows

were exposed to each of the three treatments during two periods of 3 or 4 weeks. Average daily yields of milk (adjusted to a fat content of 4 per cent) per cow for the three treatments were: basal ration, 20.0 lb.; basal ration + urea, 21.6 lb.; and basal ration + soybean oil meal, 22.8 lb. Although all cows were allowed more digestible nutrients than the minimum level suggested by Morrison (61), the cows fed the soybean oil meal-supplemented ration consumed 0.4 lb. more digestible nutrients daily than did the urea-fed cows. Cows fed the basal ration produced significantly less milk than those fed the supplemented rations. The feeding of the rations containing soybean meal resulted in the production of significantly more milk than that of the ration containing urea.

Since 2.5 per cent of the concentrate mixture used in these studies consisted of urea, this substance contributed more than one-third of the nitrogen of the total ration. In a subsequent double-reversal type of experiment, Willett *et al.* compared concentrate mixtures containing 2.5 and 1.25% of urea with a ration containing soybean meal. The experimental periods were of 4 weeks duration. Milk yields were similar (26.5 to 27.1 lb. per cow daily) regardless of the ration fed.

The small differences in favor of rations containing conventional protein feeds over those containing urea were more pronounced in double-reversal trials (1, 98) than in continuous-feeding trials (1, 80). Willett *et al.* suggested that this may mean that cows require some time to become adapted to the ingestion of urea.

The results of experiments (1, 80, 98) previously described are not in agreement with those reported by Bartlett and Blaxter (8). The latter workers conducted a series of field trials involving 274 cows on 12 farms. The addition of decorticated peanut cake to a concentrate mixture containing 12.9% crude protein to increase the protein content to 17.9% resulted in an increased milk yield during a 6-week feeding period. However, the milk yield of cows receiving the same basal ration supplemented with enough urea to provide 17.9% protein equivalent was not significantly greater than that of cows fed the basal concentrate (12.9% crude protein).

Although the differences obtained were statistically significant, the average yields of milk were very similar. For the four treatments employed the average daily milk production during the 6-week period and the decline in milk production per cow during the period, respectively, were: low-protein, 22.5 and 2.8 lb.; low-protein + urea, 22.4 and 2.7 lb.; normal protein, 22.5 and 2.0 lb.; and normal protein + urea, 23.0 and 2.4 lb.

When urea was fed in quantities greater than 3 oz. per cow daily or when it was added to rations of adequate protein content, milk production was depressed. Bartlett and Blaxter indicated that this effect of urea may be a result of toxicity arising from absorbed ammonia. Recently Clark *et al.* (19) found that ammonium hydroxide injected intravenously in sheep does not produce symptoms characteristic of urea toxicosis.

The findings of Bartlett and Blaxter (8) led them to conclude that urea has a protein-sparing action when added to a low-protein diet in small quantity, but when fed at levels needed to justify its use economically, urea is a milk-production depressant.

Although it is difficult to determine the causes for the lack of success experienced by these authors in the feeding of urea, the composition of the rations used was considerably different from those employed by workers (80, 93) in the United States. The concentrates contained wheat middlings, corn, extracted palm kernel, rice meal, molasses, and decorticated peanut cake, and the roughage portion of the ration contained three or more of the following feeds: hays, oat straw, bean straw, mangels, and beet pulp. The ratio of concentrates to roughages fed appears to have been similar to that employed by other workers. The roughage feeds available on some of the cooperating farms provided somewhat more than enough crude protein for maintenance. It seems possible, also, that the feeding periods may have been too brief to allow the animals to become adjusted to urea ingestion.

In another field trial conducted during a 210-day period, it was found that the milk yield and the rate of body weight changes of cows fed urea-containing rations were similar to those of cows fed a control ration containing conventional protein sources (34).

From an examination of the various experiments it must be concluded that no constant value can be assigned to the protein-replacement value of urea, possibly because of the many factors apparently controlling its utilization. In well controlled experiments in which significant numbers of cows have been fed during periods of considerable length, the value of urea nitrogen for milk production and for the maintenance of body weight was only slightly inferior to the nitrogen of common protein-rich feeds when fed at levels providing as much as 25% of the nitrogen of the total ration.

The use of urea to raise the protein equivalent of cereal-grain concentrates for cows from 9 or 10% to as much as 17 or 18% appears to be feasible from the physiological standpoint. Assuming that urea nitrogen is metabolically equivalent to the nitrogen of conventional protein sources, 14 lb. of urea and 86 lb. of cereal grains will replace the protein furnished by approximately 100 lb. of cottonseed or soybean meal, 115 lb. of linseed meal, or 145 lb. of distillers' dried corn grains. However, since urea has no energy value for animals, the amounts of cereal grains combined with 14 lb. of urea would have to be increased to 100 to 145 lb. in order to replace both the protein and digestible nutrients provided by the amounts of the four protein supplements listed.

Pregnant and lactating ewes. The amount and kinds of rations fed during gestation and lactation influence the amount of weight gained by ewes during pregnancy, as well as the birth and weaning weights of lambs and the fleece weights of ewes. Under usual farm conditions, rations resulting in a gain in weight of 20 to 30 lb. per ewe during pregnancy generally are considered to be sufficient to yield a healthy lamb and to result in satisfactory milk production.

Several tests of the value of urea in rations for pregnant or lactating ewes

have been made. In an experiment (74) in which ewes were fed 2 lb. of prairie hay and 1 lb. of a pelleted feed composed of corn, cottonseed meal, starch, and molasses and containing approximately 10% crude protein, an average weight of 18 lb. was gained during the gestation period. Supplementation of the ration with sufficient urea to increase the level of protein equivalent in the pelleted feed to about 14% resulted in a gain of 25.6 lb. A similar gain (26.9 lb.) was made by ewes fed pellets in which sufficient cottonseed meal was substituted at the expense of starch to furnish approximately 14% crude protein. The average birth weights of lambs produced by ewes fed the three rations were 9.8, 10.6, and 10.4 lb., respectively.

Similar results were obtained in subsequent experiments conducted by Pope *et al.* (72, 73). In addition, lambs born of ewes fed the control pellets (10.3% crude protein) gained 20.3 lb. during the first 42 days following birth, whereas gains of 24.3 and 25.1 lb. were obtained during the same period by lambs produced by ewes fed urea-containing pellets (15.6% protein equivalent) and high-cottonseed meal pellets (15.3% crude protein), respectively (72). Two pounds of prairie hay were fed to each ewe daily. During pregnancy and lactation, 1 and 2 lb., respectively, of the pelleted concentrates (containing corn, molasses, cottonseed meal, and, in one case, urea) were fed per ewe daily. No significant differences were found among the birth weights of the lambs, the fleece weights, or changes in weight of the ewes during lactation. Essentially the same results, with the exception of a lighter fleece weight in ewes fed the low-protein ration than in those receiving the two high-nitrogen rations, were obtained in a later experiment (73) conducted in a manner similar to that of the earlier tests.

The experiments conducted at the Oklahoma Station (72, 73, 74) showed that supplementation of concentrate mixtures containing 10% crude protein with enough urea to provide 15% protein equivalent yielded satisfactory results with pregnant and lactating ewes.

Practically the same weight gains were made during pregnancy by ewes fed brome grass hay supplemented with *(a)* soybean meal, *(b)* soybean meal, dehydrated alfalfa and 5% of urea, or *(c)* soybean meal, wheat middlings, and 10% of urea to provide equal intakes of nitrogen (39). Fleece weight was unaffected by the source of nitrogen. On pasture, the average daily weight gains of lambs suckling ewes which had received the three supplements were 0.42, 0.43, and 0.46 lb., respectively. Insufficient data were reported to determine whether enough nitrogen would have been provided by the supplements without the addition of urea.

A comparison was made of six different sources of nitrogen (linseed oil meal, field peas, dehydrated alfalfa, lactalbumin, urea, and urea + inorganic sulfur) in pelleted isocaloric rations fed to range ewes (97). From 3 weeks before breeding until 6 weeks after lambing, rations containing 7.8% protein equivalent were fed. The rations employed during the remainder of pregnancy and during lactation contained 11% protein equivalent. No influence of source of nitrogen upon wool yield was observed. Although the ewes receiving

the urea + surfur supplement produced less wool than those fed other sources of nitrogen, this effect was attributed to the low consumption of feed by this group. No differences in birth weights of lambs were observed; however, lambs from ewes fed lactalbumin and dehydrated alfalfa were heavier at 6 weeks of age than lambs born of ewes receiving the other nitrogen sources.

Recent evidence supports the viewpoint that urea may be used satisfactorily in the rations of pregnant and lactating ewes to provide as much as 33% of the required nitrogen.

SUMMARY

Prior to 1937 the protein-sparing action of urea was recognized, but it was not always demonstrated. Subsequent experiments have indicated that the duration of many of the early experiments was too brief to provide reliable results and that the nature of the diet to which urea was added influenced the degree to which urea was utilized.

Experiments dealing with nitrogen-balance, ruminal-ingesta composition, body composition, and isotopic tracers have been employed to demonstrate that urea nitrogen is converted to and/or stored as protein nitrogen. The conversion to protein is mediated by the microorganisms of the rumen and reticulum. Subsequent digestion of the bacteria and protozoa avails the host animal of the protein contained in the microorganisms.

The nature of the diet to which urea is added influences the growth and multiplication of ruminal bacteria and, therefore, the extent to which ruminants are able to utilize urea nitrogen. A low level of true protein and a high level of starch in the ration favors urea utilization. Highly soluble and readily hydrolyzable protein in the ration tends to depress the utilization of urea nitrogen, as bacteria appear to prefer protein nitrogen. Sugars and cellulose are inferior to starch as sources of energy for ruminal microorganisms. Since urea is soluble in water and rapidly hydrolyzed, the need for available carbohydrates at the time of ingestion is indicated. Sugars disappear from the rumen too rapidly and cellulose is made available too slowly to satisfy the needs of bacteria. In in vitro experiments it has been found that media containing moderate amounts of both readily available and complex carbohydrates support a satisfactory conversion of urea nitrogen to protein.

Although many of the results obtained in in vitro experiments have been verified in vivo, the fact that the characteristics and kinds of microorganisms are considerably different after even relatively short periods of incubation than they are at the beginning of an incubation period indicates that the application of in vitro findings to feeding practice may be misleading.

The age of the ruminant appears to influence the degree to which urea is utilized. Calves as young as 2 months of age have been shown to use some urea nitrogen. However, faster growth rates have been reported for older calves. In experiments in which urea nitrogen has been compared with the nitrogen of conventional high-protein feeds, urea nitrogen has generally been at least slightly inferior. Less difference between the two forms of nitrogen has

been found, however, in the response of milking cows than in that of growing cattle. Also, less difference has been observed in the weight gains of older steers than in those of fattening calves. With sheep, urea seems to be utilized more efficiently by mature ewes than by growing lambs.

Additions of methionine or sulfur (elemental or sulfate forms) have improved the retention of nitrogen by lambs fed urea-containing rations. The improvement effected by methionine may be explained on the basis that the protein synthesized by the ruminal microorganisms of sheep is deficient in this amino acid. When diets devoid of sulfur were fed to lambs, urea nitrogen was not utilized. An improvement in nitrogen retention by sheep has resulted from sulfur supplementation of urea-containing diets having less than 0.1% of sulfur. The needs of sheep for sulfur appear to be considerably more critical than those of cattle, probably because of wool production. Supplementation of cattle rations containing 0.1% or somewhat less of sulfur with methionine or sulfates has not resulted in any marked improvement in urea utilization. More extensive experimentation is required to determine the sulfur requirements of ruminants, the availability of the various forms of sulfur and the level of sulfur present in the feedstuffs produced in different areas. Although a definite conclusion cannot be drawn at this time, it is indicated that the kinds of rations to which urea would be added in practice contain an adequate amount of sulfur for satisfactory utilization of urea unless the roughage feeds used are markedly deficient. Since the sulfur content of crops may be influenced by soil fertilization practices, it seems possible that under certain conditions this element may be deficient in the feed.

Elements other than sulfur may influence the utilization of urea nitrogen, as has been suggested by the results of experiments conducted in vitro. There is no evidence, however, that when urea-containing rations are fed, mineral elements are needed in quantities greater than those required for satisfactory performance of animals consuming diets containing conventional protein supplements.

A problem concerning the utilization of urea which seems to require more attention is that of reducing still further the wastage of ammonia. Since urea is rapidly hydrolyzed, it appears that research dealing with the rendering of urea more slowly soluble and, thereby, possibly making the release of ammonia more gradual may be a fruitful approach.

A summary of the results of several experiments in which urea provided from 40 to 70% of the nitrogen required by dairy calves 4 months of age or older showed that body weight was gained at a rate of 82 to 88% of that of heifers fed rations supplemented with an equivalent amount of nitrogen as cottonseed meal, casein, peanut meal, or soybean oil meal and tuna fish meal. Cereal grains or other high-starch feeds composed one-third or more of these rations. Responses of a similar magnitude have been secured with beef calves. Although in some experiments no difference has been found in the rate of gain of growing cattle fed urea as compared with those consuming common high-

protein feeds, it must be concluded from the data considered as a whole that urea is somewhat inferior to conventional supplements as a source of nitrogen for growth.

The value of urea for fattening is less definite than that for growth. The results of a number of experiments suggest that urea nitrogen is approximately equivalent to the nitrogen of conventional supplements for fattening cattle when urea provides 20 to 25% of the nitrogen of the total ration. However, the value of urea for fattening calves is somewhat less than that for fattening older cattle. The value of urea for fattening appears to be less when urea is fed at levels higher than 25% of the total nitrogen. In a well conducted experiment, the gain in weight of steer calves fed a ration of which urea composed 50 to 60% of the total nitrogen was 70% of that of calves receiving a ration supplemented with casein. It is interesting, though, that the gain in calories of the urea-fed animals was 81 per cent of that of the casein-fed calves. These data suggest that, from the standpoint of energy stored, body weight may not be a completely satisfactory index of fattening. There is some evidence that a level of 1% of urea in the concentrate rations of fattening calves may be sufficiently unpalatable to reduce feed intake. An examination of recently secured data indicates that the nitrogen of urea satisfactorily replaces up to 25% of the required nitrogen for fattening beef cattle.

Urea has not been a consistently effective substitute for protein in the rations of fattening lambs. Some data indicate that up to 25% of the nitrogen in rations containing 12% protein equivalent may be satisfactorily supplied by urea. The feeding of urea at levels providing more than 25% of the total nitrogen or rations of more than 12% protein equivalent results in poor utilization of the excess urea nitrogen.

Urea is a satisfactory substitute for about one-third of the nitrogen of rations for pregnant or lactating ewes.

The results of long-time experiments with appreciable numbers of cows demonstrate that from the standpoint of milk yield and the maintenance of body weight there is no significant difference between the value of urea nitrogen (fed at levels up to 27% of the required nitrogen) and the nitrogen of high-protein supplements. When urea was fed at these levels no abnormal effects were found in reproductive behavior, composition of the milk, or general health. From a practical feeding standpoint, urea may safely compose up to 3% of the concentrate ration or up to 1% of the total ration of milking cows.

Small quantities of urea undiluted by feed and introduced suddenly into the digestive tract result in a rapid onset of toxicosis. However, a relatively large quantity of urea may be consumed without ill effects if it is uniformly mixed in the concentrate portion of the ration. When urea is uniformly mixed in feeds and fed at levels not exceeding those which successfully replace protein in rations, toxicosis will not occur. It has been reported that cattle refuse to consume enough feed containing high levels of urea to be harmed. This is believed to be due to the unpalatability of urea.

When urea is fed at levels most satisfactorily effecting protein replacement,

palatability of the ration does not appear to be reduced noticeably. There is some evidence for the improvement of the palatability of urea-containing rations by molasses and/or cobalt salts.

Since urea has no energy value for animals, urea-containing feeds must be fed at a slightly higher rate in order to provide both nitrogen and digestible nutrients in quantities equivalent to that provided by conventional feedstuffs.

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REFERENCES

(1) Archibald, J. G. Feeding Urea to Dairy Cows. Mass. Agr. Expt. Sta. Bull. 406. 1943.

(2) Arias, C., Burroughs, W., Gerlaugh, P., and Bethke, R. M. The Influence of Different Amounts and Sources of Energy upon *in vitro* Urea Utilization by Rumen Microorganisms. J. Animal Sci., **10**: 683-692. 1951.

(3) Baker, F. Normal Rumen Microflora and Microfauna of Cattle. Nature, **149**: 220. 1942.

(4) Baker, F. Microbial Synthesis and Autolysis in the Digestive Tract of Herbivora. Nature, **149**: 582-583. 1942.

(5) Baker, M. L. The Substitution of Distillers' Wheat Dried Grains or Urea for Soybean Oil Meal for Wintering Heifer Calves. Nebr. Agr. Expt. Sta. Fifty-eighth Ann. Rpt., p. 69. 1944.

(6) Baker, M. L. The Use of Urea for Fattening Yearling Steers. Nebr. Agr. Expt. Sta. Fifty-eighth Ann. Rpt., pp. 71-72. 1944.

(7) Baker, M. L. The Substitution of Distillers' Wheat Dried Grains, Urea, or Ground Rye for Soybean Oil Meal for Wintering Steer Calves. Nebr. Agr. Expt. Sta. Fifty-eighth Ann. Rpt., p. 72. 1944.

(8) Bartlett, S., and Blaxter, K. L. The Value of Urea as a Substitute for Protein in the Rations of Dairy Cattle. I. Field Trials with Dairy Cows. J. Agr. Sci., **37**: 32-44. 1947.

(9) Bartlett, S., and Cotton, A. G. Urea as a Protein Substitute in the Diet of Young Cattle. J. Dairy Research, **9**: 263-272. 1938.

(10) Bell, M. C., Gallup, W. D., and Whitehair, C. K. Utilization by Steers of Urea Nitrogen in Rations Containing Different Carbohydrate Feeds. J. Animal Sci., **10**: 1037. 1951. (Abs.)

(11) Block, R. J., and Stekol, J. A. Synthesis of Sulfur Amino Acids from Inorganic Sulfate by Ruminants. Proc. Soc. Exptl. Biol. Med., **73**: 391-394. 1950.

(12) Block, R. J., Stekol, J. A., and Loosli, J. K. Synthesis of Sulfur Amino Acids from Inorganic Sulfate by Ruminants. II. Synthesis of Cystine and Methionine from Sodium Sulfate by the Goat and by the Microorganisms of the Rumen of the Ewe. Arch. Biochem., **33**: 353-363. 1951.

(13) Briggs, H. M., Gallup, W. D., Darlow, A. E., Stephens, D. F., Dinning, J., and Campbell, W. D. Urea as a Partial Protein (Nitrogen) Supplement for Beef Cattle. Okla. Agr. Expt. Sta. Misc. Publ. MP-11: 26-30. 1947.

(14) Briggs, H. M., Gallup, W. D., Darlow, A. E., Stephens, D. F., and Kinney, C. Urea as an Extender of Protein When Fed to Cattle. J. Animal Sci., **6**: 445-460. 1947.

(15) Briggs, H. M., Gallup, W. D., Dinning, J. S., Darlow, A. E., Stephens, D. F., and Campbell, W. D. The Efficiency of Urea as a Protein Extender. Okla. Agr. Expt. Sta. Misc. Publ. MP-13: 19-23. 1948.

(16) Briggs, H. M., Gallup, W. D., Heller, V. G., and Darlow, A. E. Urea as an Extender of Protein When Fed to Lambs. J. Animal Sci., 7: 35-40. 1948.

(17) Burroughs, W., Arias, C., DePaul, P., Gerlaugh, P., and Bethke, R. M. *In vitro* Observations Upon the Nature of Protein Influences Upon Urea Utilization by Rumen Microorganisms. J. Animal Sci., 10: 672-682. 1951.

(18) Burroughs, W., Latona, A., DePaul, P., Gerlaugh, P., and Bethke, R. M. Mineral Influences Upon Urea Utilization and Cellulose Digestion by Rumen Microorganisms Using the Artificial Rumen Technique. J. Animal Sci., 10: 693-705. 1951.

(19) Clark, R., Oyaert, W., and Quin, J. I. Studies on the Alimentary Tract of the Merino Sheep in South Africa. XXI. The Toxicity of Urea to Sheep Under Different Conditions. Onderstepoort J. Vet. Research, 25: 73-92. 1951.

(20) Dinning, J. S., Briggs, H. M., Gallup, W. D., Orr, H. W., and Butler, R. Effect of Orally Administered Urea on the Ammonia and Urea Concentration in the Blood of Cattle and Sheep, with Observations on Blood Ammonia Levels Associated with Symptoms of Alkalosis. Am. J. Physiol., 153: 41-46. 1948.

(21) Dowe, T. W., Arthaud, V. H., Gregory, K., and Baker, M. L. Wheat Bran and Urea as Supplements for Fattening Cattle. Nebr. Agr. Expt. Sta. Sixty-third Ann. Rpt., p. 96. 1950.

(22) Duncan, C. W., Huffman, C. F., and Agrawala, F. P. Rumen Synthesis of Protein and Amino Acids in the Bovine on Natural and Purified Rations. J. Dairy Sci., 35: 505. 1952. (Abstract)

(23) Ehrenberg, P., Nitsche, H., and Gaffert, H. Further Experiments on Feeding Amidschnitzel to Milk Cows (Translated title). Z. Tierernähr. Futtermittelk., 2: 115-133. 1939.

(24) Ehrenberg, P., Nitsche, H., and Muller, J. Report on Protein Substitutes in Feeding Experiments at Bettlern (Translated title). Z. Tierernähr. Futtermittelk., 1: 33-71. 1938.

(25) Fingerling, G., Hientzsch, B., Kunze, H., and Reifgerst, K. Substitution of Urea for Proteins in Cattle Feed (Translated title). Landwirtsch. Vers.-Stat., 128: 221-235. 1937.

(26) Gallup, W. D., Pope, L. S., and Whitehair, C. K. Ration Factors Affecting the Utilization of Urea Nitrogen by Lambs. J. Animal Sci., 11: 621-630. 1952.

(27) Garrigus, U. S., Mitchell, H. H., Hale, W. H., and Albin, J. S. The Value of Elemental Sulfur in a Methionine Deficient Sheep Ration. J. Animal Sci., 9: 656-657. 1950. (Abs.)

(28) Hagemann, O. Contribution to the Knowledge of Protein Metabolic Products (Urea) in the Animal Organism (Translated title). Landwirtsch. Jahrb., 20: 261-291. 1891.

(29) Hamilton, T. S., Robinson, W. B., and Johnson, B. C. Further Comparisons of the Utilization of Nitrogen of Urea with That of Some Feed Proteins by Sheep. J. Animal Sci., 7: 26-34. 1948.

(30) Harris, L. E., and Mitchell, H. H. The Value of Urea in the Synthesis of Protein in the Paunch of the Ruminant. I. In Maintenance. J. Nutrition, 22: 167-182. 1941.

(31) Harris, L. E., and Mitchell, H. H. The Value of Urea in the Synthesis of Protein in the Paunch of the Ruminant. II. In Growth. J. Nutrition, 22: 183-196. 1941.

(32) Harris, L. E., Work, S. H., and Henke, L. A. The Utilization of Urea and Soybean Oil Meal Nitrogen by Steers. J. Animal Sci., 2: 328-335. 1943.

(33) Hart, E. B., Bohstedt, G., Deabald, H. J., and Wegner, M. I. The Utilization of Simple Nitrogenous Compounds Such as Urea and Ammonium Bicarbonate by Growing Calves. J. Dairy Sci., 22: 785-798. 1939.

(34) Hastings, W. H. The Use of Urea in Commercial Dairy Feeds. J. Dairy Sci., 27: 1015-1021. 1944.

(35) Johnson, B. C., Hamilton, T. S., Mitchell, H. H., and Robinson, W. B. The Relative Efficiency of Urea as a Protein Substitute in the Ration of Ruminants. J. Animal Sci., 1: 236-245. 1942.

(36) JOHNSON, B. C., HAMILTON, T. S., ROBINSON. W. B., AND GAREY, J. C. On the Mechanism of Non-protein Nitrogen Utilization by Ruminants. J. Animal Sci., 3: 287-298. 1944.

(37) JONES, I. R., AND HAAG, J. R. Utilization of Non-protein Nitrogen by Dairy Heifers. J. Dairy Sci., 29: 535-536. 1946. (Abs.)

(38) JONES, I. R., HAAG, J. R., AND WESWIG, P. H. The Relation of Sulfur Compounds to Lactation in Ruminants. J. Dairy Sci., 35: 503. 1952. (Abs.)

(39) JORDAN, R. M. Effect of Urea on Pregnant Ewes. J. Animal Sci., 11: 768. 1952. (Abs.)

(40) KIRK, W. G. Urea and Cottonseed Meal in the Fattening Ration. J. Animal Sci., 11: 769. 1952. (Abs.)

(41) KLOSTERMAN, E. W., BOLIN, D. W., LASLEY, E. L., AND DINUSSON, W. E. The Effect of Methionine Supplementation upon the Utilization of Pea and Alfalfa Proteins by Sheep. J. Animal Sci., 10: 439-446. 1951.

(42) KREBS, K. Value of the Amides in the Feeding of Cattle. Historical Consideration of the Development of the Amide Question, Critical Evaluation of the State of Our Present Knowledge (Translated title). Biedermanns Zentr. B. Tierernähr., 9: 394-507. 1937.

(43) LENKEIT, W., AND BECKER, M. The Fate of the Urea of "Amidflocken" in the Rumen. (Translated title). Z. Tierernähr. Futtermittelk., 1: 97-101. 1938.

(44) LOFGREEN, G. P., LOOSLI, J. K., AND MAYNARD, L. A. The Influence of Protein Source Upon the Nitrogen Retention by Sheep. J. Animal Sci., 6: 343-347. 1947.

(45) LONG, R. A., POPE, L. S., DARLOW, A. E., MACVICAR, R. W., AND CAMPBELL, W. D. Optimum Protein Levels and Substitutes for Cottonseed cake for Fattening Steer Calves. Okla. Agr. Expt. Sta. Misc. Publ. MP-27: 100-105. 1952.

(46) LONG, R. A., POPE, L. S., DARLOW, A. E., MACVICAR, R. W., ROSS, O. B., AND CAMPBELL, W. D. A Study of Various Substitutes for Corn and Cotton-seed Cake for Fattening Beef Calves. Okla. Agr. Expt. Sta. Misc. Publ. MP-22: 85-89. 1951.

(47) LOOSLI, J. K. Meeting the Sulfur Requirements of Ruminants. Feed Age, 2: 44-45, 58. 1952.

(48) LOOSLI, J. K., AND HARRIS, L. E. Methionine Increases the Value of Urea for Lambs. J. Animal Sci., 4: 435-437. 1945.

(49) LOOSLI, J. K., AND MCCAY, C. M. Utilization of Urea by Young Calves. J. Nutrition, 25: 197-202. 1943.

(50) LOOSLI, J. K., WILLIAMS, H. H., THOMAS, W. E., FERRIS, F. H., AND MAYNARD, L. A. Synthesis of Amino Acids in the Rumen. Science, 110: 144-145. 1949.

(51) MANGOLD, E. The Suitability and Digestibility of Some New Feeding Stuffs Containing Urea (Translated title). Forsuchungsdienst, 3: 606-608. 1937.

(52) MCDONALD, I. W. The Role of Ammonia in Ruminal Digestion of Protein. Biochem. J., 51: 86-90. 1952.

(53) MCNAUGHT, M. L. The Utilization of Non-protein Nitrogen in the Bovine Rumen. 7. Qualitative and Quantitative Study of the Breakdown of Carbohydrate Which Accompanies Protein Formation in Bovine Rumen Contents During *in vitro* Incubation. Biochem. J., 49: 325-332. 1951.

(54) MCNAUGHT, M. L., AND SMITH, J. A. B. Nitrogen Metabolism in the Rumen. Nutrition Abstr. and Revs., 17: 18-31. 1947-48.

(55) MCNAUGHT, M. L., SMITH, J. A. B., HENRY, K. M., AND KON, S. K. The Utilization of Non-protein Nitrogen in the Bovine Rumen. 5. The Isolation and Nutritive Value of a Preparation of Dried Rumen Bacteria. Biochem. J., 46: 32-36. 1950.

(56) MILLER, J. I., AND MORRISON, F. B. The Nutritive Value for Farm Animals of the Proteins Furnished by Common Protein-rich Supplements and by Typical Legume and Non-legume Rations. Cornell Agr. Expt. Sta. Fifty-first Ann. Rpt., p. 91. 1938.

(57) MILLER, J. I., AND MORRISON, F. B. The Relative Efficiency for Ruminants of the Protein Furnished by Common Protein Supplements. J. Animal Sci., 1: 353. 1942. (Abstract)

(58) Miller, J. I., Morrison, F. B., and Maynard, L. A. Relative Efficiency for Growing Lambs of Protein in Rations Supplemented by Soybean Oil Meal, Linseed Meal or Corn Gluten Meal. J. Agr. Research, **54**: 437-448. 1937.

(59) Mills, R. C., Booth, A. N., Bohstedt, G., and Hart, E. B. The Utilization of Urea by Ruminants as Influenced by the Presence of Starch in the Ration. J. Dairy Sci., **25**: 925-929. 1942.

(60) Mills, R. C., Lardinois, C. C., Rupel, I. W., and Hart, E. B. Utilization of Urea and Growth of Heifer Calves with Corn Molasses or Cane Molasses as the Only Available Carbohydrate in the Ration. J. Dairy Sci., **27**: 571-578. 1944.

(61) Morrison, F. B. *Feeds and Feeding.* The Morrison Publishing Co., Ithaca, N. Y. 21st ed. 1951.

(62) Morrison, S. H., Deal, J. F., and Dalton, H. L. Urea Supplemented with Inorganic Sulfates versus Cottonseed Meal as the Chief Source of Protein For Young Dairy Steers. (Personal Communication). 1952.

(63) Nehring, K. Feeding Trials of Urea Supplements (Amidschnitzel) for Milk Cows. Forschungsdienst, **7**: 86-98. 1939.

(64) Nehring, K., and Schramm, W. The Question of Substituting Amides for Proteins. VII. The Influence of Urea and Glycine on the Protein Exchange and Progress of Growing Sheep (Translated title). Z. Tierernähr. Futtermittelk., **2**: 201-238. 1939.

(65) Nehring, K., and Schramm, W. The Question of Substituting "Amides" for Proteins. VIII. The Utilization of the "Amides" from Molasses and "N Straw" and "N Schnitzel" by Growing Ruminants (Translated title). Z. Tierzücht. Züchtungsbiol., **54**: 238-259. 1943.

(66) Nehring, K., and Schramm, W. Effect of Ammonium Bicarbonate and Urea on the Protein Metabolism of Lambs Receiving Green Feeds (Translated title). Biedermanns Zentr. (B) Tierernähr., **15**: 241-257. 1943.

(67) Owen, E. C., Smith, J. A. B., and Wright, N. C. Urea as a Partial Protein Substitute in the Feeding of Dairy Cattle. Biochem. J., **37**: 44-53. 1943.

(68) Pearson, R. M., and Smith, J. A. B. The Utilization of Urea in the Bovine Rumen. I. Methods of Analysis of the Rumen Ingesta and Preliminary Experiments *in vivo*. Biochem. J., **37**: 142-148. 1943.

(69) Pearson, R. M., and Smith, J. A. B. The Utilization of Urea in the Bovine Rumen. 2. The Conversion of Urea to Ammonia. Biochem. J., **37**: 148-153. 1943.

(70) Pearson, R. M., and Smith, J. A. B. The Utilization of Urea in the Bovine Rumen. 3. The Synthesis and Breakdown of Protein in Rumen Ingesta. Biochem. J., **37**: 153-164. 1943.

(71) Pope, L. S., Baker, F., Gallup, W. D., and Whitehair, C. K. Urea and Cottonseed Meal as Supplements for Lamb-fattening Rations. Okla. Agr. Expt. Sta. Misc. Publ. MP-**27**: 53-56. 1952.

(72) Pope, L. S., Gallup, W. D., and Read, D. C. Urea in Rations for Pregnant and Lactating Ewes. J. Animal Sci., **11**: 773-774. 1952.

(73) Pope, L. S., Gallup, W. D., and Whitehair, C. K. The Value of Urea in Rations for Ewes During Gestation and Lactation. Okla. Agr. Expt. Sta. Misc. Publ. MP-**27**: 49-53. 1952.

(74) Pope, L. S., Whitehair, C. K., Bell, M. C., Tidwell, P. W., Bonner, M. C., and Gallup, W. D. The Use of Urea in Rations for Cattle and Sheep. Okla. Agr. Expt. Sta. Misc. Publ. MP-**22**: 59-62. 1951.

(75) Reed, F. M., Moir, R. J., and Underwood, E. J. Ruminal Flora Studies in Sheep. I. The Nutritive Value of Rumen Bacterial Protein. Australian J. Sci. Research, **2**: 304-317. 1949.

(76) Richter, K., and Herbst, J. Effects of Urea and Glycocoll as Protein Substitutes in the Feeding of Milk Cows (Translated title). Landwirtsch. Jahrb., **86**: 22-51. 1938.

(77) Ross, O. B., MacVicar, R. W., and Stephens, D. F. Feeding Trials with Mineral and Protein Supplements for Two- and Three-year-old Steers Wintering on Dry Grass. Okla. Agr. Expt. Sta. Bull. No. B-359. 1950.

(78) Ross, O. B., Stephens, D. F., MacVicar, R. W., Campbell, W. D., and Cross, F. B. A Comparison of Dried Sweet Potatoes, Mungbeans and Corn and a Comparison of Urea Pellets. Ground Mungbeans and Cottonseed Meal, for Fattening Calves. Okla. Agr. Expt. Sta. Misc. Publ. MP-11: 39-44. 1947.

(79) Ross, O. B., Stephens, D. F., MacVicar, R. W., Campbell, W. D., and Cross, F. B. A Comparison of Dried Sweet Potatoes, Ground Mungbeans and Corn and a Comparison of Urea Pellets, Ground Mungbeans, and Cottonseed Meal for Fattening Steer Calves. Okla. Agr. Expt. Sta. Misc. Publ. MP-13: 48-52. 1948.

(80) Rupel, I. W., Bohstedt, G., and Hart, E. B. The Comparative Value of Urea and Linseed Meal for Milk Production. J. Dairy Sci., 26: 647-664. 1943.

(81) Schmidt, J., and Kliesch, J. Studies on Identical Cattle Twins with Special Consideration of the Amide Question (Translated title). Züchtungskunde, 14: 193-204. 1939.

(82) Schmidt, J., and Kliesch, J. Amide Feeding Study on Identical Cattle Twins (Translated title). Züchtungkunde, 14: 401-412. 1939.

(83) Schmidt, J., Kliesch, J., and Kaempffer, A. Further Studies of the Protein-sparing Effect of Feeding Urea Supplement to Milk Cows (Translated title). Forschungsdienst, 4: 439-443. 1937.

(84) Smith, J. A. B., and Baker, F. The Utilization of Urea in the Bovine Rumen. 4. The Isolation of the Synthesized Material and the Correlation Between Protein in Synthesis and Microbial Activities. Biochem. J., 38: 496-505. 1944.

(85) Starks, P. B., Hale, W. H., Garrigus, U. S., and Forbes, R. M. The Utilization of Elemental Sulfur and Urea Nitrogen by Growing Lambs on a Purified Ration. J. Animal Sci., 11: 776. 1952. (Abstract)

(86) Thomas, W. E., Loosli, J. K., Ferris, F. H., Williams, H. H., and Maynard, L. A. Synthesis of Amino Acids in the Rumen. Federation Proc., 8: 398. 1949.

(87) Thomas, W. E., Loosli, J. K., Williams, H. H., and Maynard, L. A. Purified Diets for Studying Amino Acid Requirements of Ruminants. J. Animal Sci., 7: 534-535. 1948. (Abstract)

(88) Thomas, W. E., Loosli, J. K., Williams, H. H., and Maynard, L. A. The Utilization of Inorganic Sulfates and Urea Nitrogen by Lambs. J. Nutrition, 43: 515-523. 1951.

(89) Turk, K. L., Morrison, F. B., and Maynard, L. A. The Nutritive Value of the Proteins of Alfalfa Hay and Clover Hay When Fed Alone and in Combination with the Proteins of Corn. J. Agr. Research, 48: 555-570. 1934.

(90) Watson, C. J., Davidson, W. M., and Kennedy, J. W. The Nutritive Value of Nitrogenous Compounds for Ruminants. II. The Formation of Body Protein from Urea Labelled with the Isotope N^{15}. Sci. Agr., 29: 185-188. 1949.

(91) Watson, C. J., Kennedy, J. W., Davidson, W. M., Robinson, C. H., and Muir, G. W. The Nutritive Value of Nitrogenous Compounds for Ruminants. 1. The Nutritive Value of Urea as a Protein Supplement. Sci. Agr., 29: 173-184. 1949.

(92) Wegner, M. I., Booth, A. N., Bohstedt, G., and Hart, E. B. The "*in vitro*" Conversion of Inorganic Nitrogen to Protein by Microorganisms from the Cow's Rumen. J. Dairy Sci., 23: 1123-1129. 1940.

(93) Wegner, M. I., Booth, A. N., Bohstedt, G., and Hart, E. B. Preliminary Observations on Chemical Changes of Rumen Ingesta with and without Urea. J. Dairy Sci., 24: 51-56. 1941.

(94) Wegner, M. I., Booth, A. N., Bohstedt, G., and Hart, E. B. The Utilization of Urea by Ruminants as Influenced by the Level of Protein in the Ration. J. Dairy Sci., 24: 835-844. 1941.

(95) Weinstein, L., and McDonald, A. The Effect of Urea, Urethane, and Other Carbamates on Bacterial Growth. Science, 101: 44-45. 1945.

(96) Weir, W. C., and Rendig, V. V. Studies on the Nutritive Value for Lambs of Alfalfa Hay Grown on a Low Sulfur Soil. J. Animal Sci., 11: 780-781. 1952.

(97) Whiting, F., and Slen, S. B. The Effect of Quality and Quantity of Protein Upon the Lamb and Wool Production of Mature Range Ewes. J. Animal Sci., 11: 781. 1952. (Abs.)

(98) Willett, E. L., Henke, L. A., and Maruyama, C. The Use of Urea in Rations for Dairy Cows Under Hawaiian Conditions. J. Dairy Sci., 29: 629-637. 1946.

(99) Willman, J. P., Morrison, F. B., and Klosterman, E. W. Lamb Feeding Experiments. Cornell Agr. Expt. Sta. Bull. 834. pp. 1-62. 1946.

(100) Wohlbier, W., and Windheuser, C. Metabolism Experiments with Amidschnitzel (Translated title). Landwirtsch. Vers.-Sta. 129: 101-109. 1938.

(101) Woodward, T. E., and Shepherd, J. B. Corn Silage with the Addition of Urea and Its Feeding Value. J. Dairy Sci., 27: 648-649. 1944. (Abs.)

(102) Work, S. H., and Henke, L. A. The Value of Urea as a Protein Supplement Replacement for Dairy Heifers. Proc. Am. Soc. Animal Production. pp. 404-406. 1939.

(103) Work, S. H., Hamre, C. J., Henke, L. A., and Harris, L. E. A Note on the Effect on the Kidneys and Livers of Feeding Urea to Steers Fattening in Dry Lot and on Pasture. J. Animal Sci., 2: 166-169. 1943.

(104) Zuntz, N. Observations on the Digestion and Nutritive Value of Cellulose. (Translated title). Pflügers. Arch. f. Physiol., 49: 477-483. 1891.

CRYSTALLINE VITAMIN B_{12} REQUIREMENT OF THE YOUNG DAIRY CALF [1]

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Williams and Knodt (18) and Wallace *et al.* (16) advocated the inclusion of animal protein, particularly dried skimmilk, in milk substitutes for young dairy calves. Rusoff and Haq (11, 12) found that the addition of an animal protein factor (APF) supplement to the ration of calves or the injection of calves with vitamin B_{12} did not have a beneficial effect. Williams and Knodt (19, 20) and Bloom and Knodt (1) published similar results concerning the addition of an APF supplement to a milk replacement for calves. Hibbs and Pounden (5) fed an APF supplement to dairy calves which had been fed milk for 7 to 9 weeks and found no significant beneficial effect attributable to the supplement. Johnson *et al.* (6) stated in a preliminary report that the calf requires vitamin B_{12}. Draper *et al.* (3) reported a study of vitamin B_{12} deficiency in calves maintained on a "synthetic milk" diet in which the protein source was "alpha" protein, an isolated soybean protein.

In this investigation an attempt was made to determine the crystalline vitamin B_{12} requirement of the young dairy calf.

EXPERIMENTAL

Twenty-three Holstein and Brown Swiss male and female calves were used in this study. The calves were permitted to remain with their dams for 48 hours after parturition. The synthetic milk ration was first fed at 3 days of age and was continued for 42 days. The calves were fed twice daily by nipple pail at a rate calculated to meet the nutrient allowances for dairy calves as recommended by the National Research Council (8). The rate of dry matter feeding was 1.7 lb. per day for each 100 lb. of body weight. The formula for the basal ration is shown in Table 1 and the chemical analysis is presented in Table 2. Groups I, II, III, IV, and V received 0, 10, 20, 40, and 80 γ of crystalline vitamin B_{12} respectively, per kilogram of dry matter consumed. In addition to the basal ration, each calf received *(a)* a capsule containing 70,000 I.U. of vitamin A (shark liver oil), 10,000 I.U. of vitamin D (Viosterol), and 150 mg. of mixed tocopherols (concentrate) at the time it was placed on experiment and

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TABLE 1

Composition of the basal ration

	(%)
"Alpha" protein[a]	29.5
DL methionine	0.5
Glucose (Cerelose)	25.0
Corn syrup	20.0
Lactose	10.0
Soya lecithin	2.0
Lard	8.0
Salt mixture[b]	5.0

[a] "Alpha" protein was washed twice with tap water.

[b] Salt mixture was composed of 40 parts $CaHPO_4 \cdot 2H_2O$, 8 MgO, 7 KH_2PO_4, 5 K_2HPO_4, 5 NaCl, 5 KCL, 10 K_2SO_4, 1.98 $FeC_6H_5O_7 \cdot 5H_2O$, 0.04 $MnSO_4 \cdot H_2O$, 0.04 $CuSO_4 \cdot 5H_2O$, 0.04 $CoSO_4 \cdot 7H_2O$ and 0.05 KI.

TABLE 2

Chemical composition of the basal ration

	(%)		
Moisture	73.69	P *(%)*	0.22
Protein	8.13	Mg *(%)*	0.74
Crude fiber	0.00	K *(%)*	0.24
Ether extract	2.75	Mn *(mg./kg.)*	3.44
Ash	1.67	Co *(mg./kg.)*	2.45
Nitrogen-free extract	13.76	Fe *(mg./kg.)*	86.30
Ca	0.25	Cu *(mg./kg.)*	6.15

at weekly intervals thereafter; and *(b)* a daily dosage of 40 mg. thiamine hydrochloride, 20 mg. calcium pantothenate, 20 mg. riboflavin, 20 mg. pyridoxine hydrochloride, 20 mg. nicotinic acid, 20 mg. *p*-aminobenzoic acid, 10 mg. 2-methylnaphthoquinone, 0.04 mg. biotin, 200 mg. inositol, 0.42 mg. folic acid, and 3 g. choline chloride.

The synthetic milk was prepared by a modification of the procedure of Wiese *et al.* (17). Because of the limited refrigeration facilities available, the synthetic milk was prepared once or twice weekly and stored as a liquid concentrate. The basal ration was prepared by adding 81 g. of $NaHCO_3$, 27 g. of $Ca(OH)_2$, 40 g. of DL methionine, and 5.3 lb. of "alpha" protein, which had been washed twice previously with tap water, to 39.6 lb. of water at approximately 60° C. with constant agitation with a heavy-duty electric stirrer. Stirring was continued for 15 to 20 minutes to insure complete mixing before the soya lecithin and lard were added. The glucose, corn syrup, lactose, and salt mixture then were added to the protein-fat suspension. The synthetic milk concentrate was stirred continually until completely mixed and then homogenized at 3,000 p.s.i. The synthetic milk thus prepared contained 26.3 per cent dry matter and was stored under refrigeration in this form. At feeding time one part of the concentrate was added to about 1.5 parts of hot water, and the milk was fed at 85 to 95° F.

Evaluation of the vitamin B_{12} requirement of the dairy calf was based upon daily observations on the health and general appearance, growth rate and efficiency of feed utilization, blood analyses, and post-mortem examinations. Observations were made at least once per day with regard to the appetite,

general condition, consistency of the feces, and the general reaction of the animals. An accurate record of the feed consumption and refusal was maintained. Each calf was weighed on the day it was placed on experiment and on the 3rd, 7th, 10th, 14th, 17th, 21st, 24th, 28th, 31st, 35th, 38th, and 42nd days of the experimental period. Feed adjustments were made once per week according to the changes in body weight. Jugular blood samples were collected from each calf once weekly. Determinations of hemoglobin, hematocrit, and red blood cell counts were made on the whole blood by the methods of Sanford *et al.* (13), and Wintrobe (21), respectively. The blood plasma was analyzed for calcium (14), inorganic phosphorus (2), magnesium (4) and ascorbic acid (9). Post-mortem examinations were conducted on all animals which died during the trial or were sacrificed at the end of the experimental period. Histological sections were made of selected organs from representative animals and of any organ or tissue which appeared abnormal on gross inspection.

RESULTS AND DISCUSSION

A preliminary experiment (7) showed that "alpha" protein contained a factor which acted as an antivitamin of thiamin in the calf, but the antivitamin activity could be removed after the protein had been washed twice with water. The sodium content of the unwashed "alpha" protein was 520 mg. per 100 g. of protein. The ingestion of excessive quantities of sodium salts may have antivitamin properties, but this point was not investigated.

Within 4 to 5 days after the calves were placed on the experimental ration, the feces became very fluid and black, but the diarrhea did not seem to be infectious or injurious.

The average daily rate of growth and efficiency of feed utilization of the five experimental groups are shown in Table 3. The average daily gain in body weight of the calves in groups I, II, III, and IV were –0.10, 0.20, 0.20, and 0.65 lb. per day, respectively. The growth curves are shown in Figure 1. The incomplete data obtained on group V were assumed to be due to a deficiency or toxic factor (not the antivitamin factor) in the "alpha" protein fed to this group of calves. This group was started on experiment later than those in the four other groups and was fed a different lot of "alpha" protein. Draper *et al.* (3) also expressed a belief that "alpha" protein was deficient in a factor(s) other than vitamin B_{12}, because some of their deficient calves did not respond to vitamin B_{12} therapy. This deficiency or toxic factor is believed to be the same factor encountered in this investigation.

As indicated by the growth records of the calves in group I, (Table 3) this ration apparently was deficient in vitamin B_{12} (Fig. 1). Two of the three calves died before the 6-week experimental period was completed. In general, the calves appeared to be in normal health for the first 10 days of the trial but lost weight thereafter, had poor appetites, exhibited very poor condition, and showed general muscular weakness. The calves seemed to possess very little resistance to disease, as evidenced by the occurrence of bronchitis and pneumonia throughout the experimental period. The remaining calf in this

TABLE 3

Average daily gains in body weight over a 42-day period and the efficiency of feed utilization of calves maintained on a vitamin B_{12}-free synthetic milk ration and the ration supplemented with vitamin B_{12}

Group	No. of calves[a]	Av. starting wt.	Av. daily gain		Increase over starting wt.	Gain/lb. of D.M. consumed
		(lb.)	*(lb.)*	*(Range)*	*(%)*	*(lb.)*
I	3[b]	98	−0.10	−5.5 to −13.5	−9.8	.....
II	3	98	0.20	−10 to 32	8.5	0.12
III	3[c]	99	0.20	−12 to 11	8.4	0.13
IV	4[d]	94	0.65	22 to 30	28.8	0.40
V	10[e]	100	Incomplete data			

[a] All calves were male Holstein calves, except one Brown Swiss male in group I, and one Holstein female in group II.
[b] Two calves died, one the 16th and one on the 30th day.
[c] One died on the 36th day.
[d] One died on the 42nd day.
[e] All calves died within 13 days.

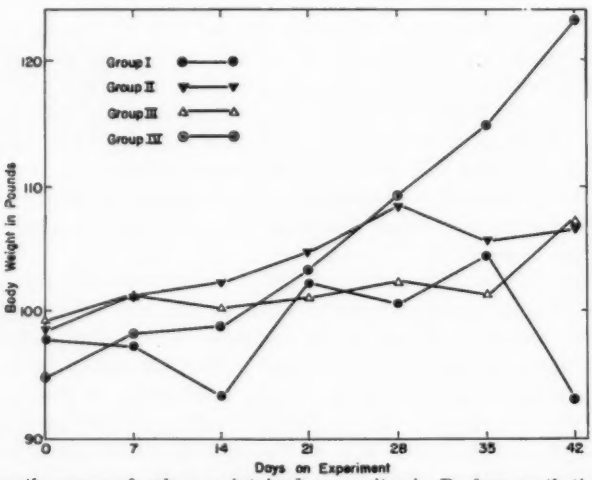

FIG. 1. Growth curves of calves maintained on a vitamin B_{12}-free synthetic milk ration (group I) and the ration supplemented with various amounts of vitamin B_{12}. Groups II, III, and IV received 10, 20 and 40 γ of vitamin B_{12} per kilogram of dry matter consumed as a supplement to the synthetic milk ration.

group was killed at the end of the trial. All three carcasses were in very poor flesh and the kidneys were somewhat enlarged and spotted with white foci ranging up to 3 mm. in diameter. The lymph nodes were enlarged and congested.

The basal ration used in this experiment was essentially the same as that formulated by Draper *et al.* (3) and the symptoms of vitamin B_{12} deficiency manifested by the calves parallel those reported by these investigators, except for the failure to observe the white-spotted kidney condition. They reported also that calves could be raised to 12 weeks of age without cessation of growth,

whereas the vitamin B_{12}-deficient calves used in this work ceased to grow after 3 or four weeks of age.

When the basal ration of the calves in groups II and III was supplemented with 10 and 20 γ of crystalline vitamin B_{12} per kilogram of dry matter consumed, none of the calves showed any of the symptoms of vitamin B_{12} deficiency exhibited by the calves in group I, other than suboptimal growth. The supplementation of the basal ration with 40 γ of vitamin B_{12} resulted in more nearly satisfactory growth (group IV). The general condition of all of the calves in this group was excellent throughout the experiment. One calf was sacrificed at the end of the trial, but none of the symptoms observed in previous trials was observed. One of the outstanding symptoms manifest by vitamin B_{12}-deficient calves was the lack of appetite. The average number of refusals of feed by the calves in groups I, II, III, and IV was 13, 7, 5, and 5, respectively, for the 42-day period. Figures 2 and 3 show representative calves

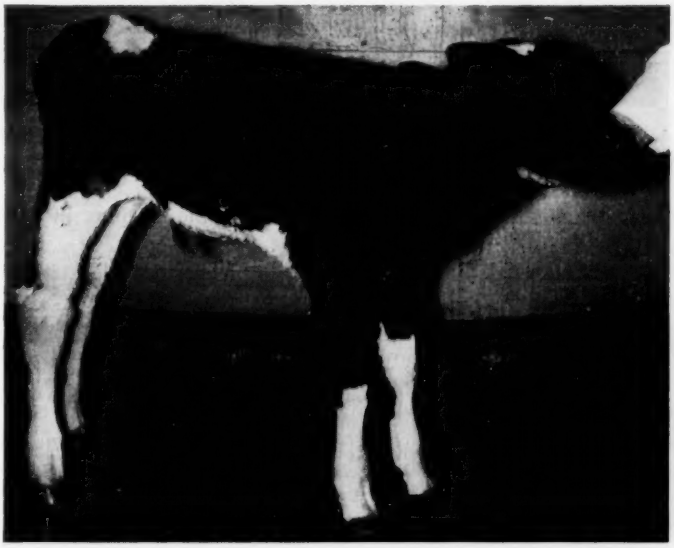

FIG. 2. Representative calf in group I at 6 weeks of age which received the vitamin B_{12}-free synthetic milk ration.

from groups I and IV at 6 weeks of age. The superior general appearance and condition of the calf shown in Figure 3 is readily apparent.

The levels of hemoglobin concentration, red blood cell volumes, and red blood cell counts in the vitamin B_{12}-deficient calves were consistently higher than those obtained from the calves on the supplemented ration. A plausible explanation of this phenomenon is that the calves were slightly dehydrated with a concurrent hemoconcentration. The ingestion of vitamin B_{12}, on the

FIG. 3. Representative calf in group IV at 6 weeks of age which received the synthetic milk ration plus 40 γ of vitamin B_{12} per kilogram of dry matter consumed.

other hand, did not appear to have any effect on the calcium, inorganic phosphorus, magnesium, or ascorbic acid content of the plasma.

The calves in group V were placed on experiment to determine whether the requirement of the dairy calf was higher than 40 γ of vitamin B_{12} per kilogram of dry matter consumed. All of the calves in this group died within the first 13 days of the trial and all showed similar growth patterns and general condition. On about the 5th day the calves began to lose body weight because of perfusive diarrhea. Post-mortem examination showed that most of the calves had slight congestion and hemorrhage of the intestinal tract, a yellowish liver, and an enlargement of the kidneys and gall bladder. The addition of either lysine or additional methionine to the ration had no favorable effect on the health of the calves. The only variation in the basal ration was the use of a different lot of "alpha" protein.

Two groups of four albino rats, averaging 69 g. in weight, then were used to study the relative nutritive value of the two lots of "alpha" protein by the rat growth method. The rations were the same as the basal ration fed to the calves except for the "alpha" protein. The rats were maintained on experiment for 6 weeks. The rats in group 1 received the original "alpha" protein and the rats in group 2 received the second lot of "alpha" protein. The growth curves for the two groups are presented in Figure 4. There was a marked difference between the rates of growth after the first 15 days. The rats in group 1 gained in body weight at a faster rate than those in group 2. At the end of the trial the rats in group 2 were losing weight, were in poor condition, and

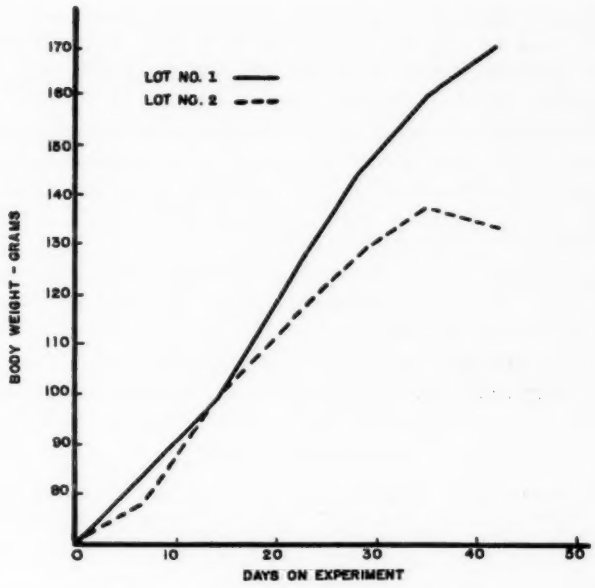

FIG. 4. Growth curves of rats fed two different lots of "alpha" protein. Group 1 received the first lot and group 2 received the second lot.

had poor appetites, whereas the rats in group 1 were in excellent condition and showed no evidence of a deficiency or a toxic factor in the ration.

The results of this investigation indicate that the young dairy calf requires a dietary source of crystalline vitamin B_{12} when a non-animal source of protein is incorporated in a synthetic milk ration. The requirement appears to be not more than 40 γ per kilogram of dry matter consumed. Rusoff and Haq (11, 12) and others (1, 19, 20), however, were unable to show any beneficial effect from the addition of vitamin B_{12} to various calf rations. The feeding of milk and animal proteins early in life probably satisfied the vitamin B_{12} requirement of the calves used in those studies. The requirements for the chick (15) and baby pig (10) have been found to be 15 and 20 γ per kilogram of ration, respectively. In this experiment, the calves fed these amounts of vitamin B_{12} showed none of the symptoms of a vitamin B_{12} deficiency, appeared to be in excellent general condition and health, and made satisfactory gains in body weight for calves maintained on synthetic milk rations.

SUMMARY

Twenty-three newborn dairy calves were allotted to five experimental groups and fed an "alpha" protein synthetic milk ration deficient in vitamin B_{12}. The basal ration was supplemented with 0, 10, 20, 40, and 80 γ of crystalline vitamin B_{12} per kilogram of consumed dry matter in an attempt to establish the vitamin

B_{12} requirement of the young dairy calf. The average daily gains of calves on the 0, 10, 20, and 40 γ levels were −0.10, 0.20, 0.20, and 0.65 lb. per day, respectively. Incomplete data were obtained for group V because of a toxic factor present in this lot of "alpha" protein.

It was found that the young dairy calf requires vitamin B_{12} when a non-animal protein is the only source of protein in a synthetic ration. Growth cessation, lack of appetite, general poor condition, muscular weakness, and a white-spotted kidney condition were symptoms found to be associated with vitamin B_{12} deficiency in the young dairy calf.

Preliminary results indicated that the crystalline vitamin B_{12} requirement of the dairy calf is more than 20 γ but not more than 40 γ of vitamin B_{12} per kilogram of dry matter consumed.

Evidence was obtained also which indicates that "alpha" protein is not always satisfactory as a source of protein for the young dairy calf.

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REFERENCES

(1) Bloom, S., and Knodt, C. B. The Value of Vitamin B_{12}, DL-Methionine, Penicillin, and Aureomycin in Milk Replacement Formulae for Dairy Calves. J. Animal Sci., 10: 1039-1040. 1951.

(2) Briggs, A. P. A Modification of the Bell-Doisy Phosphate Method. J. Biol. Chem., 53: 13-16. 1922.

(3) Draper, H. H., Sime, J. T., and Johnson, B. C. A Study of Vitamin B_{12} Deficiency in the Calf. J. Animal Sci., 11: 332-340. 1952.

(4) Duncan, C. W., Huffman, C. F., and Robinson, C. S. Magnesium Studies in Calves. I. Tetany Produced by a Ration of Milk or Milk with Various Supplements. J. Biol. Chem., 108: 35-44. 1935.

(5) Hibbs, J. W., and Pounden, W. D. The Performance of Rumen Inoculated Calves Fed a High Roughage Ration with and without APF Supplement. J. Animal Sci., 9: 659. 1950.

(6) Johnson, B. C., Nevens, W. B., and Mitchell, H. H. B-Vitamin Nutrition of the Dairy Calf Studied with "Synthetic Milk" Diets. J. Dairy Sci., 34: 506. 1951.

(7) Lassiter, C. A. Crystalline Vitamin B_{12} Requirement of the Young Dairy Calf. Ph.D. Thesis. Mich. State College Library, East Lansing. 1952.

(8) Loosli, J. K., Huffman, C. F., Petersen, W. E., and Phillips, P. H. Recommended Nutrient Allowances for Domestic Animals. 3. Recommended Nutrient Allowances for Dairy Cattle. Natl. Research Council, Washington, D. C., 1950.

(9) Mindlin, R. L., and Butler, A. M. The Determination of Ascorbic Acid in Plasma; A Macromethod and a Micromethod. J. Biol. Chem., 122: 673-686. 1938.

(10) Nesheim, R. O., and Johnson, B. C. Quantitative Vitamin B_{12} Requirement of the Pig. Federation Proc., 9: 366-367. 1950.

(11) Rusoff, L. L., and Haq, M. O. Is A.P.F. of Value in a Calf Starter for Calves Weaned from Milk at an Early Age? J. Dairy Sci., 33: 379-380. 1950.

(12) Rusoff, L. L., and Haq, M. O. Effect of Vitamin B_{12} (A.P.F.) on the Growth of Calves Weaned from Milk at an Early Age. J. Animal Sci., 10: 331-334. 1951.

(13) SANFORD, A. H., SHEARD, C., AND OSTERBERG, A. E. The Photelometer and Its Use in the Clinical Laboratory. Am. J. Clin. Path., 3: 405-420. 1933.

(14) SHOHL, A. T. The Effect of Hydrogen-ion Concentration upon the Determination of Calcium. J. Biol. Chem., 50: 527-536. 1922.

(15) STOKSTAD, E. L. R., JUKES, T. H., PIERCE, J., PAGE, A. C., JR., AND FRANKLIN, A. L. The Multiple Nature of the Animal Protein Factor. J. Biol. Chem., 180: 647-654. 1949.

(16) WALLACE, H. D., LOOSLI, J. K., AND TURK, K. L. Milk Substitutes for Young Dairy Calves. J. Dairy Sci., 32: 709. 1949.

(17) WIESE, A. C., JOHNSON, B. C., MITCHELL, H. H., AND NEVENS, W. B. Synthetic Rations for the Dairy Calf. J. Dairy Sci., 30: 87-94. 1947.

(18) WILLIAMS, J. B., AND KNODT, C. B. The Value of Milk Replacements in the Rations of Dairy Calves. J. Dairy Sci., 32: 986-992. 1949.

(19) WILLIAMS, J. B., AND KNODT, C. B. APF Supplements in Milk Replacements for Dairy Calves. J. Dairy Sci., 33: 380. 1950.

(20) WILLIAMS, J. B., AND KNODT, C. B. APF Supplements in Milk Replacements for Dairy Calves. J. Animal Sci., 10: 144-148. 1951.

(21) WINTROBE, M. M. *Clinical Hematology.* Lea & Febiger, Philadelphia, Pa. pp. 201-211. 1942.

THE DIGESTIBILITY AND INTERRELATIONSHIPS OF VARIOUS CARBOHYDRATE FRACTIONS OF PASTURE HERBAGE AND A RESOLUTION OF THE COMPONENTS OF CRUDE FIBER AND NITROGEN-FREE EXTRACT[1]

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The system of proximate analysis which was introduced by Henneberg in 1863 involves the determination of crude protein, crude fiber, nitrogen-free extract, ether extract, and ash and is still used today in essentially the same form as that first introduced (2). Undoubtedly, the measurement of crude fiber and nitrogen-free extract was an attempt to divide the feed constituents into digestible and indigestible portions. Any portion of a feed-stuff that could not be extracted or dissolved by successive treatments with weak acid and weak alkaline solutions was intended to represent the indigestible portion. Actually the nitrogen-free extract fraction contains portions of relatively indigestible materials, such as lignin, and the crude fiber fraction is digested to a very considerable extent by ruminants. Crampton (5) pointed out that in pasture herbage the crude fiber may be just as digestible and, often, even more digestible than the nitrogen-free extract.

Neither the crude fiber nor the nitrogen-free extract fractions represent any precise chemical constituent or group of constituents. They vary in composition with different plant species, stages of maturity of the plant, and with the conditions under which they are determined.

In the ninety years since Henneberg introduced this system of feed analysis, much progress has been made in the evaluation of feedstuffs. No doubt, the determinations of crude fiber and nitrogen-free extract have aided materially in this progress; however, it is surprising that methods which do not measure precise chemical groups of constituents have been used without major modifications for such a long period of time; especially, when Henneberg, himself, realized their shortcomings (2, 13).

Other systems of analysis for the carbohydrate group and associated substances have been proposed, such as the determination of cellulose, hemicelluloses, and lignin (6) and a similar scheme in which lignin is determined indirectly (7). These methods have not been widely employed.

A system of analysis is needed that will indicate the nutritive value of feed materials more accurately than the system of proximate analysis. This ex-

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periment was conducted to determine the content of various carbohydrate constituents and their changes, interrelations and digestibilities at different stages of plant maturity.

EXPERIMENTAL

Constituents of pasture forage (predominantly timothy) were investigated in hand-feeding and grazing trials during the vegetative, boot-to-early head, and full-bloom stages of growth. Two groups of three steers (two Holsteins and one Hereford) were used in these experiments. Continuously through the season, one group was fed definite quantities of clipped forage and the other group grazed forage of the same source. At three intervals, the feces of both groups were totally collected in fecal collection bags.

The contents of lignin, hemicelluloses, cellulose, pectins, starch, total sugars and the proximate constituents were determined in the feces and clipped herbage samples. It is recognized that some of the constituents measured may have been also included either completely or in part in other constituent groups. The apparent digestibility of the various constituents or groups of constituents was determined. The interrelations existing among the quantities of these constituents and the relations between the quantities of each constituent and the digestibility of the dry matter were examined by the calculation of correlation coefficients.

An attempt was made to account for the dry matter content of the forages and feces by the summation of the quantities of the constituents believed to consist of one substance.

The following analytical methods were employed to measure the various constituents: lignin, method of Ellis, Matrone, and Maynard (9) as modified by Thacker (23); hemicelluloses, method of Weihe and Phillips (25); cellulose, method of Crampton and Maynard (6); pectins, methods of Carré and Haynes (4) and Nanji and Norman (14) with modifications (17, 25); starch and total sugars, method of Heinze and Murneek (12), and the proximate constituents, methods of Association of Official Agricultural Chemists (1). It was assumed that the crude protein determined in forages and feces contained 16 per cent nitrogen.

RESULTS AND DISCUSSION

The composition of the forages consumed and of the feces voided by animals consuming the three forages is shown in Table 1. The composition of the grazed forage was computed according to the procedure outlined by Hardison and Reid (11). The cell-wall constituents (lignin, hemicelluloses, and cellulose) of forage generally increased with advancing growth. The crude fiber content also increased with advancing growth. The pectin content of the herbage decreased with advancing growth which may mean that, as the plant matures, pectin is replaced by lignin as stated by Norman (15); however, it does not necessarily mean that pectin is a precursor of lignin but, at least, indicates a changing metabolism in the plant (3). The contents of sugars and starch did

TABLE 1

Mean composition of forages and feces (per cent of dry matter)

	Type of trial[a]	Vegetative stage				Boot-to-early head stage				Full-bloom stage			
		Forage		Feces		Forage		Feces		Forage		Feces	
Crude Protein	H	16.1		15.7	±0.21[b]	12.0		12.1	±0.35	10.4		9.8	±0.18
	G	18.0[c]	±0.76	17.3	±0.70	13.6	±0.42	13.6	±0.38	11.5	±0.92	10.7	±0.78
Crude fiber	H	25.2		24.6	±0.30	27.3		28.4	±0.53	31.8		31.0	±0.77
	G	22.8	±0.66	22.3	±0.69	25.4	±0.74	26.5	±0.81	29.4	±1.74	28.5	±1.88
Nitrogen-free extract	H	46.4		40.2	±0.34	49.4		45.5	±0.25	49.1		46.9	±0.20
	G	46.2	±0.36	39.6	±0.40	47.8	±0.35	43.5	±0.36	49.2	±0.72	46.6	±1.00
Ether extract	H	4.8		8.2	±0.21	4.3		5.7	±0.24	3.3		5.6	±0.25
	G	5.7	±0.36	9.6	±0.59	5.1	±0.21	6.6	±0.27	3.8	±0.53	6.3	±0.84
Ash	H	7.5		11.3	±0.36	6.9		8.3	±0.26	5.4		6.8	±0.24
	G	7.3	±0.22	11.2	±0.35	8.0	±0.38	9.7	±0.44	6.1	±1.02	7.9	±1.26
Cellulose	H	29.9		24.4	±0.14	32.0		29.8	±0.60	33.4		31.0	±1.31
	G	30.0	±1.66	24.3	±1.41	30.4	±0.37	28.1	±0.38	29.8	±1.88	27.5	±1.92
Hemicelluloses	H	17.2		14.9	±0.31	19.2		19.4	±0.25	20.2		18.6	±0.34
	G	18.2	±1.59	15.7	±1.40	17.8	±0.52	18.0	±0.54	23.7	±1.40	21.8	±1.42
Starch	H	8.2		1.21[d]	±0.01	7.8		0.92[d]	±0.02	7.7		0.74[d]	±0.03
	G	8.6	±0.06	1.26[d]	±0.01	6.9	±0.15	0.83[d]	±0.03	7.7	±0.05	0.73[d]	±0.01
Sugars	H	5.5		0.23	±0.01	6.8		0.20	±0.01	5.2		0.10	±0.03
	G	4.9	±1.40	0.20	±0.06	4.2	±1.19	0.12	±0.03	12.4	±0.19	0.24	±0.01
Pectins	H	2.24		1.00	±0.03	1.75		1.30	±0.19	1.13		0.86	±0.04
	G	1.59	±0.17	0.71	±0.07	1.97	±0.69	1.44	±0.50	1.12	±0.10	0.84	±0.06
Lignin	H	5.8		23.9	±0.78	6.7		20.8	±0.27	8.6		22.3	±0.27
	G	5.2	±0.14	21.3	±0.74	6.9	±0.45	21.3	±1.37	8.5	±0.24	21.8	±0.46
Undetermined matter[e]	H	2.76		—0.84	±1.73	2.55		1.48	±0.31	4.67		4.20	±0.59
	G	0.51	±3.48	—1.57	±2.07	5.13	±1.96	0.31	±1.20	—4.62	±0.72	2.19	±0.05

[a] H = hand-feeding trial; G = Grazing trial.

[b] Mean ± standard error of mean.

[c] Composition of grazed herbage was estimated [Hardison and Reid (11)].

[d] Starch content of feces was estimated on basis that starch was 96% digested.

[e] Undetermined matter represents dry matter not accounted for by summation of protein, fat, ash, cellulose, starch, sugars, pectins, and lignin.

not show any definite trend relative to growth stage. The nitrogen-free extract content of the grazed herbage increased with advancing growth but that of the hand-fed herbage was irregular.

The hemicelluloses, cellulose, crude fiber, and nitrogen-free extract contents of the feces increased as the herbage consumed became more mature. Although small amounts of sugar were determined in feces, this may have been the result of interference by non-sugar substances. The lignin content was highest in the feces resulting from the most immature forage. This suggests the possibility that a given amount of lignin may depress the digestibility of other constituents to a greater degree in mature plants than it does in immature ones, resulting in a higher concentration of lignin in feces produced from immature herbage. These results agree with the common observation that a small increase in the lignin content of forages accompanies a large decrease in the digestibility of forage (13).

A comparison of the composition of the feces yielded by hand-fed and grazing animals showed that the crude protein content was consistently higher, the crude fiber content was lower, and the nitrogen-free extract content tended to be lower in the feces of the grazing steers. These differences in composition appeared to be an expression of the preferences of grazing animals for leaves. Grazing animals selectively graze the plants available to them in a manner which has not been duplicated by manual-sampling procedures, despite many attempts to do so. For this reason the chemical analysis of the clipped forage used in this study applies only to that fed to the hand-fed steers. The procedure (11) employed to estimate the composition of grazed herbage is feasible only when conventional digestion trials accompany grazing trials on the same herbage. The composition of the grazed herbage estimated by this method was different from that of the hand-fed herbage. The amount of crude protein was higher and that of the fiber constituents was lower in grazed herbage than in the whole, clipped herbage, which supports the common belief that grazing animals tend to select the leafier, more nutritious portions of forage plants. In these trials, an attempt was made to allow the grazing steers to graze the allotted areas to the same stubble height (approximately 2 inches) as the adjacent areas from which herbage was clipped for the hand-fed steers. Although this was not entirely accomplished, it probably restricted the selective grazing tendencies of the animals.

The digestibility of the individual constituents of hand-fed and grazed herbage is given in Table 2. The dry matter digestibility of the grazed forage was calculated by the fecal-chromogen method (19). The digestibility of the constituents of grazed forage was calculated in the conventional way, using the estimated composition of the forage.

Lignin in the hand-fed forage was completely indigestible. The recovery of the ingested lignin from the feces was over 100 per cent. The lignin procedure was checked using forage samples of known lignin content from Ellis' (8) laboratory. Close agreement was obtained. No standard samples of feces were tested, but the interfering substances are assumed to have been in the

TABLE 2

Digestibility of constituents of forage hand fed and grazed (per cent)

Constituents	Vegetative stage		Boot-to-early head stage		Full-bloom stage	
	Hand fed	Grazed[a]	Hand fed	Grazed[a]	Hand fed	Grazed[a]
Dry matter	72.9 ± 0.29[b]	76.9 ± 0.44	66.3 ± 0.78	69.4 ± 1.39	58.0 ± 1.67	58.2 ± 0.50
Crude protein	73.7 ± 0.28	77.8 ± 0.47	66.0 ± 0.91	69.5 ± 1.26	60.8 ± 1.00	61.1 ± 0.72
Ether extract	53.7 ± 1.71	60.8 ± 0.84	56.1 ± 0.47	60.4 ± 1.81	30.3 ± 2.02	30.7 ± 1.26
Crude fiber	73.6 ± 0.39	77.5 ± 0.48	65.0 ± 1.43	68.0 ± 1.47	59.1 ± 2.32	59.4 ± 0.76
Nitrogen-free extract	76.5 ± 0.10	80.3 ± 0.42	69.0 ± 0.77	72.1 ± 1.28	59.9 ± 1.62	60.4 ± 0.74
Ash	58.9 ± 1.72	64.6 ± 0.75	59.5 ± 1.42	63.1 ± 1.70	47.3 ± 2.59	46.0 ± 1.01
Lignin	—11.7 ± 4.13	5.7 ± 2.01	—4.9 ± 2.64	5.3 ± 4.36	—9.0 ± 3.08	8.0 ± 2.02
Cellulose	77.8 ± 0.08	81.3 ± 0.40	68.6 ± 1.27	71.6 ± 1.30	61.0 ± 3.06	61.5 ± 0.72
Hemicellulose	76.5 ± 0.64	80.1 ± 0.42	65.8 ± 0.91	69.0 ± 1.42	61.3 ± 1.14	61.7 ± 0.72
Starch[c]	96.0	96.0	96.0	96.0	96.0	96.0
Total sugars	98.9 ± 0.10	99.0 ± 0.02	99.0 ± 0.06	99.1 ± 0.06	99.2 ± 0.27	99.2 ± 0.02
Pectins	87.8 ± 0.40	92.3 ± 2.57	75.1 ± 3.10	77.7 ± 1.04	68.3 ± 2.07	68.7 ± 0.60

[a] Estimated digestibility of constituents of grazed herbage.

[b] Mean ± standard error of mean.

[c] Digestibility of starch assumed to be 96 per cent (based upon literature and unpublished data).

feces. The digestibility of the sugars in the herbage was almost complete at all three stages of growth studied whereas that of all other constituents studied decreased progressively with advancing plant maturity.

As a means of investigating the interrelations among the herbage and fecal constituents and their relations to dry matter digestibility, correlation coefficients were computed. As the amounts of the cell-wall constituents (lignin, hemicelluloses, and cellulose) increased in the forage, the quantities of the other constituents (pectins, starch, and crude protein) generally decreased. With the small number of samples involved in this study, very high correlations were required for statistical significance. The correlations[3] of the amounts of several other forage constituents with the amount of digestible dry matter were significant[4]: crude protein, 0.945; lignin, —0.985; crude fiber, —0.966; and nitrogen-free extract, —0.882. The correlation coefficients between the quantities of crude fiber and those of nitrogen-free extract and lignin were 0.827 and 0.942, respectively. The nitrogen-free extract content was also correlated with the lignin content (0.832). A correlation coefficient of —0.836 was obtained between the amounts of hemicelluloses and pectins in the forages.

Although the composition of the feces of animals hand-fed roughages or forages has no great importance other than allowing the determination of digestion coefficients, the composition of the feces of grazing animals may be indicative of the nature of the herbage selected. In this study it was found that the amounts of crude fiber, nitrogen-free extract, hemicellulose and cellulose in the feces were highly correlated with each other and with the digestible dry matter content of the forage. The correlation coefficients between the amounts of these fecal constituents and the digestible dry matter were negative. The quantity of crude protein in the feces was correlated positively with dry matter digestibility.

An attempt was made to resolve the dry matter in feces and forages by determining the quantities of crude protein, ether extract, ash and the components of crude fiber and nitrogen-free extract. The constituents of the crude fiber and nitrogen-free extract fractions measured were: lignin, hemicelluloses, cellulose, pectins, starch, and total sugars. The percentage of the dry matter not accounted for by a summation of these values is shown as "undetermined" matter in Table 1. In this scheme, the dry matter determined differed from the actual dry matter by an average of 3.4 per cent for the forages and 1.8 per cent for the feces. The magnitude of the undetermined portion of forages was not related to growth stage, whereas the amount of undetermined dry matter in feces seemed to increase as the herbage consumed became more mature.

Since sugars and starch are generally believed to be almost completely digested (10, 24), the values obtained for feces in this experiment are probably artifacts. It is possible that, in the determination of starch, diastase acted upon some of the constituents of the hemicellulose fraction to form reducing sugars

[3] Correlations include data of hand-feeding and grazing trials.

[4] Significance levels: 5% = 0.811; 1% = 0.917.

which result in the calculation of fictitiously high values for starch. For this reason and because of the values recorded in the literature and of recent work in this laboratory in which starch was found to be about 96 per cent digestible, the feces were assigned starch values correspondent with an indigestibility of 4 per cent.

If starch is not a normal constituent of grasses, as suggested by Sullivan and Garber (22), the apparent starch measured in both forages and feces in this study may have been an artifact. However, if starch is not present, the material measured as starch in the forage may be fructosan or some other constituent that may not have been included in the hemicelluloses fraction.

Summations of the carbohydrate constituents of forage and feces computed in two ways are shown in Table 3. The sums of the amounts of cellulose, hemi-

TABLE 3

Summation of the amounts of carbohydrates in forages and feces

	Hand-fed forage	Feces from hand-fed steers	Grazed forage	Feces from grazing steers
Vegetative stage				
Sum of carbohydrates[a] *(%)*	62.98	41.79 ± 0.44[b]	63.25 ± 4.27	42.22 ± 2.62
(Fiber + NFE) — lignin *(%)*	65.77	40.77 ± 1.31	63.88 ± 1.19	40.59 ± 1.78
Boot-to-early head stage				
Sum of carbohydrates *(%)*	67.48	51.63 ± 0.35	61.30 ± 2.65	48.53 ± 1.32
(Fiber + NFE — lignin *(%)*	70.08	53.09 ± 0.59	66.30 ± 1.38	48.69 ± 2.35
Full-bloom stage				
Sum of carbohydrates *(%)*	67.68	51.34 ± 1.30	74.76 ± 3.42	51.02 ± 3.30
(Fiber + NFE — lignin *(%)*	72.33	55.60 ± 0.26	70.14 ± 2.70	53.30 ± 3.34

[a] Sum of percentages of cellulose, hemicelluloses, starch, sugars and pectins.
[b] Mean ± standard error of mean.

celluloses, starch, sugars and pectins result in values not greatly different from the sums of the contents of lignin-free crude fiber and nitrogen-free extract. This suggests that the latter value may provide an efficient accounting for the total carbohydrate content, thereby avoiding a detailed analysis. The digestibilities of the lignin-free crude fiber and nitrogen-free extract fraction and the fraction composed of cellulose, hemicelluloses, pectins, starch and sugars, respectively, at various stages of growth of the hand-fed herbages were: vegetative, 83.2 and 82.0 per cent; boot-to-early head, 74.4 and 74.2 per cent, and full-bloom, 67.8 and 68.2 per cent.

The partitioning of the carbohydrates of forages into crude fiber and nitrogen-free extract did not divide them into indigestible and digestible portions. With the forages employed in this study, these fractions were digested to about the same extent. Also, the major constituents (cellulose and hemicelluloses) of these two groups were of similar digestibilities. Cellulose and hemicelluloses constituted approximately 75 and 95 per cent of the lignin-free crude fiber and nitrogen-free extract fraction and of the total carbohydrates of forages and feces, respectively. In these experiments, the detailed

analysis for the constituents of crude fiber and nitrogen-free extract yielded very little additional information concerning the nutritive value of the herbages over that obtained from the crude-fiber, nitrogen-free extract and lignin values. Also, neither the detailed carbohydrate analysis nor the measurement of crude fiber and nitrogen-free extract from which sum the lignin content was deducted showed any advantage over determining the total carbohydrate content by difference.

Consequently, as a result of the relationships observed in this experiment, it is indicated that, in studies of forage crops not requiring a breakdown of individual carbohydrates, a measure of nutritive value may be effected by the determination of the crude protein, ether extract, ash, lignin and moisture contents. The application of these analyses is expressed by the following equation:

$$\%\ \text{total carbohydrates} = 100\% - (\%\ \text{crude protein} + \%\ \text{ether extract} + \%\ \text{ash} + \%\ \text{lignin}).$$

This analytical scheme, treating all carbohydrates as a single group and obviating the crude fiber analysis, would result in about the same amount of cost as the usual proximate analysis because of the additional analysis for lignin. However, it would result in a separation of nutrients into groups of greater biological significance than those represented by the proximate constituents.

Since lignin, as measured by the method of Ellis *et al.* (9), is generally indigestible, this approach to the evaluation of forage feeds would not interfere with the measurement of the total digestible nutrient content, which may be computed according to the following expression:

$$\%\ \text{total digestible nutrients} = \%\ \text{digestible carbohydrates} + \%\ \text{digestible crude protein} + (2.25 \times \%\ \text{ether extract}).$$

At the three growth stages examined, the total digestible nutrient values of the hand-fed herbages computed in the conventional way and in the manner described above, respectively, were: vegetative, 71.9 and 72.5 per cent; boot-to-early head, 65.3 and 65.6 per cent, and full-bloom, 56.9 and 57.7 per cent. These values for herbage of a given growth stage were not identical because of the small negative digestion coefficients obtained for lignin.

SUMMARY

The composition and digestibility of timothy-mixed grasses has been studied at three timothy growth stages (vegetative, boot-to-early head, and full-bloom). Three steers were hand-fed herbage freshly clipped from areas adjacent to those grazed at the same time by three other steers.

The pasture grasses became more unpalatable and less digestible as they matured. These changes were accompanied by changes in the chemical constituents of the plants. At successive stages of maturity, the amounts of the cell-wall constituents (lignin, cellulose, and hemicellulose) increased progressively, and those of pectins, sugars, crude protein, and ether extract decreased.

Lignin as determined in this investigation was completely undigested. Sugars

were almost completely digested in the herbage at the three growth stages studied. The digestibility of all other constituents measured decreased progressively with advancing plant maturity.

A comparison of the composition of the feces of the hand-fed and grazing steers indicated that the grazing steers selected leafier and more highly digestible portions of the plant than were consumed by the hand-fed steers.

High negative correlations were obtained between the amounts of digestible dry matter and those of lignin, crude fiber, and nitrogen-free extract in the grass. High negative correlations were observed also between the fecal contents of crude fiber, nitrogen-free extract, cellulose and hemicelluloses and the digestible dry matter content of the herbage, whereas a high positive correlation was observed between the crude protein content of feces and the digestible dry matter content of the herbage. With further study of other forages, the correlations between the amounts of fecal constituents may reveal relationships that can be used satisfactorily to estimate the digestibility of forage material.

Only small amounts of the dry matter of forages and feces were unresolved by analyses for lignin, hemicelluloses, cellulose, pectins, starch, sugars, crude protein, ether extract and ash. Although these constituents represent more or less distinct chemical groups, this detailed analysis is expensive. It would appear that, in studies of the digestibility and composition of forage feeds, the replacement of the crude fiber analysis with an analysis for lignin and the measurement of the total carbohydrates by difference would result in the partitioning of nutrient groups having more meaning biologically than the presently employed proximate analysis.

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REFERENCES

(1) Association of Official Agricultural Chemists. Official and Tentative Methods. 6th ed., pp. 404 and 762. Washington, D. C. 1945.

(2) Browne, C. A. The Origin and Application of the Term Nitrogen-free Extract in the Valuation of Feeding Stuffs. J. Assoc. Offic. Agr. Chemists, 23: 102-108. 1940.

(3) Buston, H. W. Observations on the Nature, Distribution and Development of Certain Cell-wall Constituents of Plants. Biochem. J., 29: 196-218. 1935.

(4) Carré, M. H., and Haynes, D. The Estimation of Pectin as Calcium Pectate and the Application of this Method to the Determination of Soluble Pectin in Apples. Biochem. J., 16: 60-69. 1922.

(5) Crampton, E. W. Pasture Studies. XIV. The Nutritive Value of Pasture Herbage. Some Problems in its Estimation and Some Results Thus Far Obtained at Macdonald College. Sci. Agr., 19: 345-357. 1939.

(6) Crampton, E. W., and Maynard, L. A. The Relation of Cellulose and Lignin Content to the Nutritive Value of Animal Feeds. J. Nutrition, 15: 383-395. 1938.

(7) Crampton, E. W., and Whiting, F. A Proposed Scheme of Feeding-stuffs Analysis. J. Animal Sci., 2: 378-384. 1943.

(8) Ellis, G. H. Report on Lignin and Cellulose in Plants. J. Assoc. Offic. Agr. Chemists, 32: 287-291. 1949.

(9) Ellis, G. H., Matrone, G., and Maynard, L. A. A 72 Percent H_2SO_4 Method for the Determination of Lignin and its Use in Animal Nutrition Studies. J. Animal Sci., 5: 285-296. 1946.

(10) Fraps, G. S. Digestibility by Chickens of the Constituents of the Nitrogen-free Extract of Feeds. Texas Agr. Expt. Sta. Bull. 437. 1931.

(11) Hardison, W. A., and Reid, J. T. Degree of Herbage Selection by Grazing Cattle. (To be published)

(12) Heinze, P. H., and Murneek, A. E. Comparative Accuracy and Efficiency in Determination of Carbohydrates in Plant Material. Mo. Agr. Expt. Sta. Research Bull. 314. 1940.

(13) Louw, J. G. The Relative Digestibility of the Constituents of the Carbohydrate Complex of Grasses at Successive Stages of Growth with Reference to Their Partition into Crude Fiber and Nitrogen-free Extract According to the Standard Method for Feeding Stuff Analysis. Onderstepoort J. Vet. Sci. Animal Ind., 17: 165-179. 1941.

(14) Nanji, D. R., and Norman, A. G. Studies in Pectin. Part II. The Estimation of the Individual Pectic Substances in Nature. Biochem. J., 22: 596-604. 1928.

(15) Norman, A. G. *The Biochemistry of Cellulose, the Polyuronides, and Lignin.* The Clarendon Press, Oxford. pp. 21, 70-120, 151-183, 187-188. 1937.

(16) Phillips, M., Davidson, J., and Weihe, H. D. Studies of Lignin in Wheat Straws with Reference to Lodging. J. Agr. Research, 43: 619-626. 1931.

(17) Phillips, M., Davis, B. L., and Weihe, H. D. Composition of the Tops and Roots of the Timothy Plant at Successive Stages of Growth. J. Agr. Research, 64: 533-546. 1942.

(18) Phillips, M., Miller, C. O., and Davis, R. E. Composition and Apparent Digestibility of the Carbohydrate and Other Constituents of Pea and Lima-bean Vines When Fed to Sheep. J. Agr. Research, 73: 177-187. 1946.

(19) Reid, J. T., Woolfolk, P. G., Hardison, W. A., Martin, C. M., Brundage, A. L., and Kaufmann, R. W. A Procedure for Measuring the Digestibility of Pasture Forage under Grazing Conditions. J. Nutrition, 46: 255-267. 1952.

(20) Richards, C. R., and Reid, J. T. The Use of Methoxyl Groups in Forage and Fecal Materials as an Index of the Feeding Value of Forages. J. Dairy Sci., 35: 595-602. 1952.

(21) Schryver, S. B., and Thomas, E. M. The Hemicelluloses. II. The Hemicellulose Content of Starches. Biochem. J., 17: 497-500. 1923.

(22) Sullivan, J. T., and Garber, R. J. Chemical Composition of Pasture Plants with Some Reference to the Dietary Needs of Grazing Animals. Pa. Agr. Expt. Sta. Bull. 489. 1947.

(23) Thacker, E. J. U. S. Plant, Soil and Nutrition Laboratory, Ithaca, N. Y. Unpublished Data.

(24) Thurber, F. H. Starch as a Constituent of Nitrogen-free Extract. J. Assoc. Offic. Agr. Chemists, 23: 126-131. 1940.

(25) Weihe, H. D., and Phillips, M. The Quantitative Estimation of Hemicelluloses by Direct Isolation. J. Agr. Research, 74: 77-85. 1947.

THE USE OF HEATED HOMOGENIZED MILK AS A DILUTER FOR BULL SEMEN[1]

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Boiled homogenized milk has been shown by Thacker and Almquist (4, 5) and Almquist and Thacker (1) to be a satisfactory substitute for egg yolk-citrate as a diluter for bull semen. A report by several artificial breeding organizations does not show consistent results on the use of boiled homogenized milk (2).

Since field reports (2) indicated some variation in results from use of boiled homogenized milk, two split sample trials were started in March, 1952, comparing this diluent with egg yolk-citrate. Experiment 1 was conducted at Northwest Co-op Breeders, Mount Vernon, Washington, through June 1952. Experiment 2 was conducted at Evergreen Breeders Association, Chehalis, Washington, through May 1952.

EXPERIMENTAL

Experiment 1. Homogenized milk (referred to hereafter as milk) was heated to 95° C. in a covered stainless steel double boiler, held for one minute, and then cooled in tap water. The milk was purchased daily from a local grocery store and represented the product of one distributor. Egg yolk-citrate was prepared using one part of egg yolk to one part of 3 per cent sodium citrate dihydrate. One thousand units of penicillin and 1,000 γ of streptomycin per milliliter of diluted semen were added to both the milk and the egg yolk-citrate.

Semen was collected daily, except Sundays, with all bulls on weekly collection schedules. Semen from bulls collected on Saturday morning was used within 60 hours, and semen from other bulls generally was used within 36 hours after collection. Semen from bulls collected on Saturdays was diluted with egg yolk-citrate one week and with milk the next week (hereafter referred to as alternate week). All other bulls were on a split sample basis.

Experiment 2. The homogenized milk, procured as previously described, was heated in an open Pyrex double boiler to 95° C. for 10 minutes and cooled in tap water. This method of heating most nearly approximates that used by Thacker and Almquist (5). Egg yolk-citrate for this experiment was prepared in the same manner as for experiment 1. Five hundred units of penicillin and 500 γ of streptomycin per milliliter of diluted semen were added to both the diluters.

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Semen was collected every other day with all bulls in this experiment on a 6-day collection schedule, and most of the breedings were made with semen the day of and the day after collection. All bulls were used regularly on the third day by inseminators in counties unable to receive semen the same day as collected.

Breeding efficiency for both experiments was expressed as a percentage of 60-90 day nonreturns to first and second services. Statistical methods used were according to Snedecor (2).

RESULTS AND DISCUSSION

Experiment 1. The average nonreturn for egg yolk-citrate for this experiment, as shown in Table 1, was 63.5%, as compared with 68.1% for milk. The three breeds and all bulls involved in the study responded in a like manner. Bulls which were used on weekends showed a 3% difference between egg yolk-citrate

TABLE 1
Average 60-90 day nonreturn rates for experiment 1

	Diluters					
	Egg yolk-citrate		Milk			
Group	No. serv.	% N. R.	No. serv.	% N. R.	No. bulls	No. samples
Split samples						
Guernsey	2,671	61	2,722	65	7	66
Jersey	1,087	68	1,028	72	5	66
Holstein	1,931	65	1,945	71	6	66
Total	5,689	63.5	5,695	68.1	18	198
Alternate week samples	2,141	64	2,199	67	7	78
Day of use						
Day 1[a]	1,169	66	1,102	69		
Day 2	5,564	64	5,635	69		
Day 3	1,097	60	1,157	63		
Total	7,830	63.7	7,894	68.1		

[a] Same day as collection.

and milk. Also shown in Table 1 is the change in nonreturn rate associated with age of semen in egg yolk-citrate and milk. The difference between the first and third day was 6% for both egg yolk-citrate and milk; however, the difference in favor of the milk was consistent over the 3 days.

An analysis of variance of these data, shown in Table 2, indicated that there was a highly significant difference between treatments ($P < 0.01$).

Experiment 2. The difference between egg yolk-citrate and milk in this experiment, shown in Table 3, was 2.8% in favor of the milk. This difference was also statistically significant. There was a drop in percentage nonreturn between the first and third day for egg yolk-citrate of 8% but the drop was only 4% for milk. The difference on the first day was 2% and on the third day 6% in favor of the milk.

TABLE 2

Analysis of variance of the 60-90 day nonreturns for split samples for egg yolk-citrate and milk diluters in experiment 1

Source of variation	Degrees of freedom	Mean square
Inseminator groups	1	0.0
Treatments	1	660.0[a]
Bulls	17	165.0[b]
Bulls × treatments	17	41.5
Bulls × inseminator groups	17	25.4
Inseminator groups × treatments	1	80.0
Bulls × inseminator groups × treatments	17	37.7

[a] Significant at 1% level of probability.
[b] Significant at 5% level of probability.

TABLE 3

Average 60-90 day nonreturn rates for experiment 2

	Diluters					
	Egg yolk-citrate		Milk			
Group	No. serv.	% N. R.	No. serv.	% N. R.	No. bulls	No. samples
Guernsey	574	56	632	65	3	36
Jersey	526	62	570	65	3	37
Holstein	639	67	700	67	3	35
Hereford	657	65	622	66	3	37
Day of use						
Day 1[a]	756	66	860	68		
Day 2	1,123	63	1,127	64		
Day 3	517	58	537	64		
Total	2,396	62.8	2,524	65.6	12	145

[a] Same day as collection

The two experiments reported herein differ in three respects. In experiment 1, homogenized milk was heated in a covered stainless steel double boiler to 95° C. and held for 1 minute, whereas the homogenized milk for experiment 2 was heated to 95° C. and held for 10 minutes in an open Pyrex double boiler. In experiment 1, penicillin and streptomycin were used at twice the level of that in experiment 2. The source of homogenized milk was different in that it came from a different processor in a different area of the state. Any one of these three factors could account for the differences between these two experiments. The percentage fat would vary only to the extent that the quantity exceeded the minimum legal standards of 3.25%. In experiment 1, semen diluted with milk was 4.6% higher than egg yolk-citrate (split samples) compared with 2.8% for experiment 2. The influence of differences in level of antibiotics used cannot be evaluated.

Thacker and Almquist (5) found fertility of semen diluted with boiled homogenized milk 1.3% higher compared with egg yolk-citrate containing penicillin and streptomycin. Almquist and Thacker (1) later found a 4% difference in favor of boiled homogenized milk over egg yolk-citrate when penicillin and streptomycin were used in both diluters. In both these trials homogenized milk was held for 10 minutes at 92-95° C. in an open Pyrex double

boiler, which corresponds with the method used for experiment 2 of this report. The results of the experiment extend and confirm those of Thacker and Almquist (5) and Almquist and Thacker (1).

SUMMARY

Two split sample field trials were conducted. Experiment 1 involved 198 samples from 18 bulls of three breeds on a split sample trial and 78 samples from seven bulls on alternate week trial. The average nonreturn rate for the split samples with egg yolk-citrate on 5,689 services was 63.5%, whereas the same semen diluted with boiled homogenized milk on 5,695 services was 68.1%. This difference of 4.6% is significant ($P < 0.01$). The 78 ejaculates from the seven bulls on an alternate week basis and used for 4,340 first and second services showed a 3% increase in favor of milk.

Experiment 2 involving 145 split samples from 12 bulls of four breeds and used for 4,920 first and second services showed a difference of 2.8% in favor of the boiled homogenized milk ($P < 0.01$).

REFERENCES

(1) Almquist, J. O., and Thacker, D. L. Fertility of Bull Semen Diluted With Boiled, Homogenized Milk and Boiled Skim Milk. (Abs.) J. Animal Sci., 11: 787. 1952.
(2) Proc. Ann. Meet. Natl. Assoc. Artificial Breeders. Springfield, Mo. 1952.
(3) Snedecor, G. W. *Statistical Methods.* 4th ed. Iowa State College Press, Ames. 1946.
(4) Thacker, D. L., and Almquist, J. O. Milk and Milk-Products as Diluters for Bovine Semen. (Abs.) J. Animal Sci., 10: 1082. 1953.
(5) Thacker, D. L., and Almquist, J. O. Fertility and Motility of Bovine Spermatozoa in Boiled Milk. J. Dairy Sci., 36: 173-180. 1953.

INITIAL LEVELS OF FRUCTOSE AND CITRIC ACID IN BULL SEMEN AS RELATED TO FERTILITY[1, 2]

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Bovine semen is characterized by high levels of fructose and citric acid originating from the accessory glands (4, 8). Citric acid as a constituent of semen was established through the work of Schersten, cited by Mann (7). The appearance of these constituents in the semen of the young animal has been associated with approaching sexual maturity (9). Between samples of different bulls there is considerable variation in levels and also in relative levels of the two constituents (2, 4, 6, 7, 8, 9). With successive collections over a short period of time the values for fructose and citric acid remain more constant than do numbers of sperm (6).

Fructose in semen is a principal source of energy for sperm (8, 10). Van Tienhoven *et al.* (14) found glucose preferentially utilized as compared to fructose when extraneous glucose was added. The role of citric acid is less clearly defined. Enzymes of the citric acid cycle have been shown by Lardy and Phillips (5) to be present in semen. They also found in incubation studies with bovine epididymal sperm that there was appreciable accumulation of citrate; they noted no increase with incubation of ejaculated sperm. Humphrey and Mann (4) found citrate utilization, although the amount of such citrolysis was not of the magnitude of fructolysis. Citrate is also suggested as being a contributing agent to the buffering system of semen (7).

The variability in quantity of fructose and citric acid encourages investigation to determine their relation to the variability of fertilizing capacity. Conceivably relationships to fertility might be found by two different measures: (a) levels of fructose and/or citric acid at time of ejaculation; (b) changes in amounts of either or both constituents due to metabolic activity of sperm after ejaculation.

The authors are not aware of any study in which fructose content of individual samples has been compared with fertilizing capacity of the same samples. Likewise no comparable data are available on citric acid and fertility. The primary purpose of the study reported herein was to determine the relationship of initial levels of fructose and citric acid to fertilizing capacity of semen.

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PROCEDURE

Samples were obtained from all bulls of the Northwest Co-op Breeders of Mount Vernon, Washington, for one year beginning in August 1950. Immediately after collection, a 0.5-ml. portion was transferred to a small vial and frozen. The remainder of the semen was used in routine quality evaluations and for inseminations. At weekly intervals the accumulated frozen samples were sent under refrigeration to Pullman. In a preliminary testing of techniques the handling of samples in such a manner demonstrated no appreciable changes in the constituents being studied.

TABLE 1

Average values for fructose, citric acid and nonreturn for all bulls of three breeds

Breed	Bull No.	No. of samples	Av. fructose		Av. citric acid		60-90 day nonreturn rate
			Whole semen	Plasma	Whole semen	Plasma	
			(Mg. %)	*(Mg. %)*	*(Mg. %)*	*(Mg. %)*	*(%)*
Guernsey							
	1	41	901	981	681	744	66
	2	22	731	811	735	815	68
	3	34	600	660	724	796	65
	4	20	393	452	654	756	63
	5	41	540	589	698	762	62
	6	10	661	731	855	943	61
	7	42	578	642	696	776	67
	8	27	423	462	536	588	67
	9	5	368	414	680	770	73
	10	13	432	471	521	567	67
	11	13	750	840	738	831	59
	Total	268	608	670	681	754	65
Jersey							
	12	25	452	492	717	780	70
	13	40	436	505	698	812	70
	14	20	565	614	850	925	67
	15	27	536	584	728	801	77
	16	30	456	515	683	768	72
	17	19	352	403	686	787	74
	26	6	543	615	677	762	63
	Total	167	468	523	719	806	72
Holstein							
	18	22	550	615	743	832	62
	19	38	544	607	713	796	70
	20	38	584	618	716	759	65
	21	26	551	610	898	1,000	68
	22	31	454	504	746	827	71
	23	41	534	596	812	911	71
	24	25	602	651	902	977	68
	25	7	668	747	640	721	67
	Total	228	548	603	778	859	68

When the samples arrived, they were made up to 5 ml. with the addition of distilled water. After thorough mixing, 1 ml. of the diluted semen was withdrawn by pipette and forcibly ejected into 9 ml. of cold 10% trichloroacetic acid. After they had stood 30 minutes under refrigeration, the samples were centrifuged.

Fructose determination was by the method of Roe (11) and citric acid by the method of Taussky and Shorr (13), with slight adaptation of each method

to make suitable measurement of quantities present in semen. Statistical evaluations were according to Snedecor (12).

Data on concentration of sperm and 60-90 day nonreturn rates on first and second services were available. Most breedings were made within 48 hours after collection of the semen.

Sperm concentration has an influence on the measuring of constituents that arise from the accessory glands. This is due to the fact that a sample with high concentration of sperm will have a higher proportion of the semen volume represented as cell volume. For this reason it was decided to analyze the data after adjusting for sperm concentration. Essentially, this means that the cell volume of a sample is deleted from the total volume to establish a plasma volume. Actual procedure used in this determination of cell volume has been reported (3).

RESULTS AND DISCUSSION

Of the samples on which fructose and citric acid determinations were made, 663 were used for 20 or more first and second breedings (1). These 663 included 268 from 11 Guernsey, 167 from 7 Jersey, and 228 from 8 Holstein sires. The average values of fructose, citric acid, and nonreturn rate for individual bulls are included in Table 1. Values for fructose and citric acid are expressed on total semen as well as plasma.

TABLE 2

Average values for fructose, citric acid, and nonreturn rate by months

Month	No. of samples	Av. fructose		Av. citric acid		Nonreturn rate
		Whole semen	Plasma	Whole semen	Plasma	
		(Mg. %)	*(Mg. %)*	*(Mg. %)*	*(Mg. %)*	*(%)*
Aug.	40	624	681	888	968	67
Sept.	19	635	694	804	879	66
Oct.	34	629	691	814	897	69
Nov.	41	559	623	703	784	65
Dec.	67	497	551	772	856	66
Jan.	74	569	628	673	745	69
Feb.	68	546	606	653	726	67
Mar.	65	550	611	699	780	67
Apr.	64	509	562	795	880	68
May	69	483	531	703	776	69
June	71	545	606	690	771	71
July	51	618	687	648	725	67
Total	663	552	610	724	803	68

Table 2 shows the average values for fructose, citric acid, and nonreturn rate by months. Average fructose for all samples on a whole semen basis was 552 mg. % with a standard deviation of 169. Average citric acid for all samples was 724 mg. % with a standard deviation of 192. Average values by months are also graphically portrayed in Figure 1.

There was a low order over-all highly significant correlation between initial fructose levels and nonreturn rate of —0.123. Individual breed correlations

were: Guernsey, —0.013; Jersey, —0.028; and Holstein, —0.088. The average within-breed correlation as determined by analysis of covariance was —0.037. None of these latter correlations were significant.

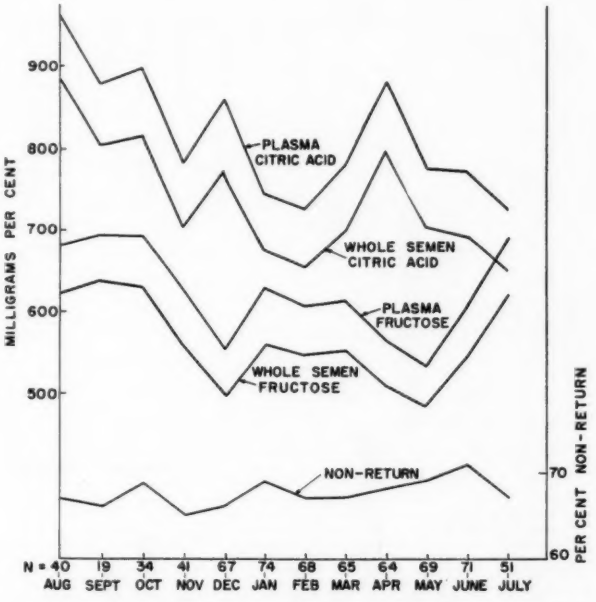

FIG. 1. Average values for fructose, citric acid, and nonreturn rate by months.

Correlations by months are shown in Figure 2. With the number of samples involved, none of the individual month correlations between fructose and nonreturn rate were significant. Ten of the months, however, showed varying degrees of negative correlation.

The over-all correlation between initial citric acid levels and nonreturn rate was only —0.021. Individual breed correlations were Guernsey, —0.111; Jersey, 0; and Holstein, —0.037. The average within-breed correlation was —0.057. The correlations by months between citric acid and nonreturn rate had a rather unusual pattern. As shown in Figure 2, there was a significant negative correlation (—0.603) in September. Data for this particular month included the smallest number of samples (19) and, furthermore, included only 3 Jersey samples, which may have given undue "breed" effect. In contrast to the fructose $\times$ nonreturn rate correlations, there were 6 months in which a positive, though nonsignificant, relationship between citric acid and nonreturn rate was present.

The view that this citric acid relation to nonreturn rate has some cyclic basis instead of purely random variation is given support by the data presented

in the lower part of Figure 2. The initial levels of fructose and citric acid are shown to be positively correlated in all but 3 months. There was significant correlation in October and April.

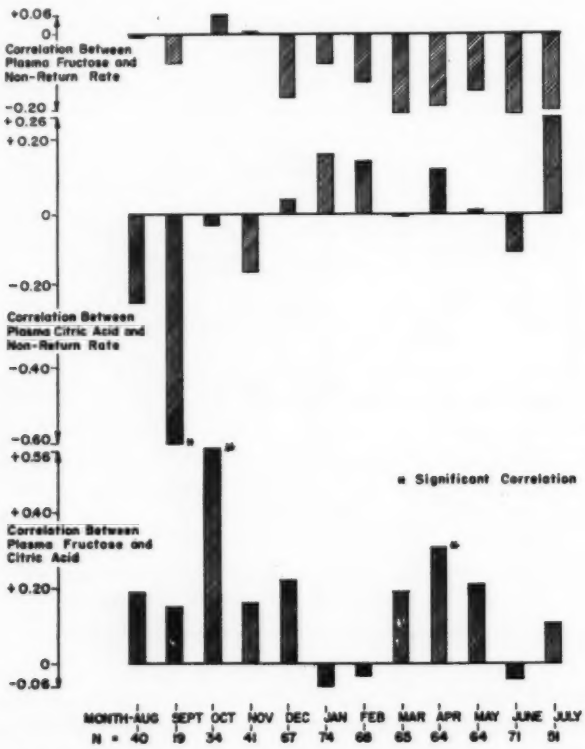

FIG. 2. Correlations between plasma fructose and nonreturn rate, between plasma citric acid and nonreturn rate by months, and between plasma fructose and citric acid.

Mann (4, 9) found fructose to appear first in the accessory glands of the castrate upon the administration of testosterone propionate, with citric acid appearing later. This would lead to the hypothesis that seasonal fluctuations in amounts of fructose and citric acid are due to variations in hormone activity. Furthermore, seasonal trends in fructose need not coincide with trends of citric acid (4, 9), even though both are produced in accessory glands under the influence of male hormones. In the data studied, lowest average levels of fructose were in December and May. Lowest average levels of citric acid were in February and July. These low months for fructose preceded the low months for citric acid by 2 months.

The same line of reasoning, however, cannot be applied to a strict lag

relation between high average levels of fructose and citric acid. Such average comparisons also neglect the influence of semen volume in that low levels per unit of semen do not necessarily mean lowest total quantity of the constituents.

For these reasons it is believed that the best approach to studying interrelationships between fructose, citric acid, and fertility is by means of correlations involving time of the year.

SUMMARY

Six hundred sixty-three semen samples used in 20 or more first and second inseminations were analyzed for initial levels of fructose and citric acid. Samples were collected over a 12-month period from Guernsey, Jersey, and Holstein bulls. There was no significant relationship between fructose or citric acid and nonreturn rate after breeds were removed.

Correlations by months showed low order, nonsignificant negative values between fructose and nonreturn rate for 10 of the 12 months. Citric acid in semen showed varying degrees of low order negative correlations during six of the months and varying degrees of low order positive correlations in the other six months. The distribution of the values was such that fructose and citric acid did not show the same relationship to nonreturn rate.

Evidence of the cyclic relationship between fructose and citric acid was indicated in that the correlations between fructose and citric acid were significantly positive in October and April but were slightly negative during January, February, and June.

REFERENCES

(1) Erb, R. E., Ehlers, M. H., Mikota, L., and Schwarz, E. The Relation of Simple Semen Quality Tests to Fertilizing Capacity of Bull Semen. Wash. Agr. Expt. Sta. Tech. Bull. 2. 1950.

(2) Flerchinger, F. H., Ehlers, M. H., Crilly, J. B., and Erb, R. E. Fructose and Citric Acid Content of Bull Semen. Proc. 32nd Ann. Meet., West. Div. Am. Dairy Sci. Assoc. Bozeman, Mont. 1951.

(3) Flerchinger, F. H., and Erb, R. E. The Conversion of Seminal Constituents to a Seminal Plasma Basis when Analyzed as Total Semen. (Abs.) J. Dairy Sci., 36: 579-580. 1953.

(4) Humphrey, G. F., and Mann, T. Studies on the Metabolism of Semen. 5. Citric Acid in Semen. Biochem. J., 44: 97-105. 1949.

(5) Lardy, H. A., and Phillips, P. H. Studies of Fat and Carbohydrate Oxidation in Mammalian Spermatozoa. Arch. Biochem., 6: 53-61. 1945.

(6) Mann, T. Fructose Content and Fructolysis in Semen. Practical Application in the Evaluation of Semen Quality. J. Agr. Sci., 38: 323-331. 1948.

(7) Mann, T. Metabolism of Semen. Advances in Enzymol., 9: 329-390. 1949.

(8) Mann, T. Studies on the Metabolism of Semen. 3. Fructose as a Normal Constituent of Seminal Plasma. Biochem. J., 40: 481-491. 1946.

(9) Mann, T., Davies, D. V., and Humphrey, G. F. Fructose and Citric Acid Assay in the Secretions of the Accessory Glands of Reproduction as Indicator Tests of Male Sex Hormone Activity. J. Endocrinol., 6: 75-85. 1949.

(10) Mann, T., and Lutwak-Mann, C. Studies on the Metabolism of Semen. 4. Aerobic and Anaerobic Utilization of Fructose by Spermatozoa and Seminal Vesicles. Biochem. J., 43: 266-270. 1948.

(11) Roe, J. H. A Colorimetric Method for the Determination of Fructose in Blood and Urine. J. Biol. Chem., 107: 15-22. 1934.
(12) Snedecor, G. W. *Statistical Methods*, 4th ed. Iowa State College Press, Ames. 1946.
(13) Taussky, H. H., and Shorr, E. A Micro Colorimetric Method for the Determination of Citric Acid. J. Biol. Chem., 169: 103-118. 1947.
(14) VanTienhoven, A., Salisbury, G. W., VanDemark, N. L., and Hansen, R. G. The Preferential Utilization by Bull Spermatozoa of Glucose as Compared to Fructose. J. Dairy Sci., 35: 637-641. 1952.

PEOPLE and EVENTS
in the Dairy Science World

A Message from the President

The American Dairy Science Association was organized to serve the dairy industry through research and teaching. The results have been improved milk production, better manufacturing procedures, closer controls of products and processes, and higher standards of quality and sanitation. A little more than half of the membership is engaged in research and teaching today; the rest of the members are active in some phase of production, processing, or servicing.

Walter V. Price

The greatest combined service effort of all members is the publishing of the Journal of Dairy Science. For nearly 36 years this Journal has published mainly original papers on fundamental and applied science. In recent years it has added a section of abstracts of the world's important dairy literature.

Now we have asked our editor, through a recent survey, to make some changes in the Journal material and organization. Subject material of research usually emphasizes important questions. This emphasis, reflected in our original papers, changes from time to time. If you have profited recently by the numerous papers on production—remember it when the emphasis turns in another direction. These trends are beyond the control of our editor and editorial board, except insofar as papers and reviews may be invited in the future. In this way, by carefully choosing subject material by outstanding workers and writers, the editor proposes to achieve, in part, more authentic articles in the popular section for which we members have voted.

Some of us feel that our important committee reports might appear in the Journal when they are thoughtfully conceived, carefully verified, and properly written for publication. Recommended analytical methods, for example, would be compared in several laboratories and data presented to indicate reliability of recommended procedures. Such reports when developed by several authorities, and properly approved, might thus become not only valuable contributions to the literature but reliable, working instructions for improved techniques.

As changes are made in the Journal to follow suggestions of the membership, take time to express your reactions to your Editor and to your Journal Management Committee. It's your Journal — written for your benefit.

Remember that increasing the scope of the Journal raises the total of our costs which already are equivalent to our income. We want more members to benefit by our services, and we need more subscriptions to help carry the increased costs.

Every member can get a member. By so doing the member helps himself — as well as others.

Walter V. Price
President, A.D.S.A.

Staff Changes at Rhode Island

J. Douglas Dodds, formerly instructor and superintendent of the Dairy Farm, has been promoted to assistant professor and dairy extension specialist at the University of Rhode Island. He replaces John Atwood, who accepted a position with the American Guernsey Cattle Club, Peterborough, N. H.

Fred Warren, a graduate of the University of Connecticut, has been appointed instructor and farm superintendent at the University of Rhode Island, effective July 1, 1953.

Florida Dairymen Honor Reaves

C. W. Reaves, extension dairyman with the University of Florida, was honored at the annual meeting of the Florida Dairy Association at Miami, June 24 to 26, by election to the "Order of Bell-Cows," the dairy leadership fraternity. Mr. Reaves' election this year makes a total of nine dairymen and professional dairy workers who have been thus honored by the Association.

News from Arizona

F. G. Harland of the staff of the Department of Dairy Husbandry, University of Arizona, will take a leave of absence commencing September 1, 1953, to complete work for a Ph.D. at Texas A. & M. College. His field of study will be in dairy cattle nutrition. Mr. Harland, a 1940 graduate of Michigan State College, has been on the staff at the University of Arizona since 1946.

The dates for the third annual Dairy Industry Conference sponsored by the Department of

Dairy Husbandry, will be February 25-26, 1954.

West Virginia Happenings

GEORGE BULKLEY of the Carnation Co., Los Angeles, Calif., was the principal speaker at the annual W.V.U. Dairy Day, June 30. Some 1,000 visitors attended the event, viewed the herds, and witnessed demonstrations on the newest methods of operating a dairy farm.

JOHN T. GODFREY, who was granted his M.S. at W.V.U. this June, has accepted a position with the I.B.M. installation in the Extension Division of the Department of Animal Husbandry at Cornell Univ.

Will Announces ADA Programs

LESTER J. WILL has announced that Bob Hope and Bob Crosby have been signed to promote a new radio and TV advertising campaign for the American Dairy Association. He stated that these programs, in addition to the regular national advertising program, will make it possible for ADA to deliver nearly 50,000,000 messages for dairy foods each week.

Milk Control in California Under Attack

A suit has been filed by the Lucerne Dairy Co. (a subsidiary of Safeway Stores) to enjoin the State Agriculture Department from setting prices in Kings County California. The Company does not question the need to protect milk producers by establishing minimum prices to farmers but feels that wholesale and retail prices should not be controlled. This probably will be a significant test case, for, regardless of outcome, it probably will be carried to the higher courts by the losing party. The future of all state milk control laws may be at stake.

Industry Conferences

The 14th Annual Michigan Dairy Manufacturers Conference will be held November 4 and 5, at the Kellogg Center, Michigan State College. Three specialized groups of dairy processors: market milk; ice cream; and butter, cheese and dried milk, will meet concurrently for the two-day session. An instructional clinic for dairy products will be conducted on Wednesday afternoon. A general banquet sponsored by the joint membership of the Detroit and Western Michigan Dairy Technology Societies is scheduled for the evening of November 4.

The University of Illinois will hold a conference for soft ice cream and ice milk manufacturers on its Urbana campus November 11 and 12.

International Show Trophies

More than $30,000 in cash prizes and 56 trophies will be awarded to exhibitors of six major breeds of cattle at the first International Dairy Show, scheduled for October 10-17 in Chicago. The International Amphitheatre, which is being prepared to house 2,500 head of cattle, will be the scene of one of the most impressive showings ever held of pure bred dairy cattle. As many as 20 prizes in one class are listed in the various breeds, with first prizes ranging up to $60. Total premiums in each breed are approximately $5,000.

A state herd class, in which cattle must be owned by not less than three exhibitors, lists premiums of $500 in each breed. Also included in each breed is a Premier Exhibitor Award and a Premier Breeder Award, to be made to the exhibitors and breeders of animals winning the most money in open single classes.

Trophies are offered by various breed associations and dairy companies. In the Ayrshire group, trophies will be awarded in three classes, and two trophies will be given for Brown Swiss. Trophies are offered for first in twelve classes in the Guernsey show; Holstein exhibitors will receive 28 trophies; Jersey, four trophies; and Milking Shorthorn, seven.

Members of 4-H and FFA Clubs will enter the Junior Show, which boasts a substantial prize list, as well as 14 trophies and a 35-mm. camera award to the grand champion in each of the six dairy breeds in the Junior Division.

Completed Theses

Ph.D. Degree:

MISCHA E. FRIEDMAN and MYROSLOW PURKO were erroneously listed in the August issue as having received Masters degrees at the University of Illinois. They received Ph.D. degrees.

60 Copies of January and February Issues Needed

If you do not make a permanent file of your Journal numbers, the Association would appreciate receiving your January and February, 1953, issues. Send your copies to P. R. Ellsworth, Secretary, A.D.S.A., The Ohio State University, Columbus, Ohio.

Report on Journal Survey

The survey on a proposed journal and a popular section in the Journal of Dairy Science was sent to members of the Association. The 546 replies received by June 6, when the returns were tabulated, were classified as follows:

47% Academic: Teaching and research, educational institutions.

9% Extension: Extension, educational institutions.

14% Commercial Research: Research in commercial laboratories.

30% Commercial: All others, including laboratory technicians, plant workers, quality control men, farmers, fieldmen, etc.

Of the 546 returns, 51.5% replied affirmatively to the question: "Do you favor . . . the

proposed new journal?" The commercial groups especially favored it, the others were inclined against it.

Introducing a popular section in the Journal of Dairy Science was favored by those who said "yes" to the new journal idea; the "no" voters also were agreeable. A decisive majority, 62% of the 546 voters, favored establishing a popular section in the Journal of Dairy Science.

Suggestions. Space was allowed on the questionnaire for "yes" voters to make suggestions to insure success of the proposed new journal. The "no" voters used the same space to give reasons for their answers. Many valuable suggestions were given for editing the proposed journal and for improving the Journal of Dairy Science. The Journal Management Committee was especially pleased with these thoughtful responses.

The results of this survey are not a mandate to start a new journal at this time. The majority of members do approve the establishment of a popular section in the Journal of Dairy Science, providing it is carefully developed in accordance with the ideals of service and accuracy of the present Journal.

Members are willing to express their views. It is hoped that frequent surveys may be used to test the reactions of readers to features in the Journal, such as: types of papers, organization of material, abstracting service, and the like. The information so obtained should be very useful as Editor Tracy pursues his course of making the Journal more valuable to all who read it.

Walter V. Price, retiring chairman,
Journal Management Committee.

Dairy Technology — and Men

A Guest Editorial

The small number of students now specializing in Dairy Technology and the shortage of superior, technically trained men in industry have invoked statements from industrialists and educators as to the causes. In certain quarters, the finger of suspicion has been pointed at the universities and the charge has been made that the curricula are antiquated, the teaching methods archaic, and the educational program lacking in breadth and depth—resulting in an inadequate training program for men who aspire to leadership in the commercial field. In contrast, others have claimed that the principal responsibility lies with the dairy industry for not using trained dairy technologists properly, and prospective students are turning to other fields which offer greater opportunities. To the majority of interested persons, it appears that both the universities and industry must share the responsibility for the situation which prevails.

I. A. Gould

It behooves those of us in education to accept the problem — to analyze our respective curricula, and make such changes as are necessary to meet the needs of industry. By all means, a boy interested in dairy technology should not be forced to take an entire series of non-related agricultural subjects, but, instead, should be directed to select subjects in science, engineering, and business administration. Furthermore, the responsibility is ours to reduce the number of "know how" courses and to incorporate more of the "know why" approach in the training of students. We must gear our teaching philosophy and methods so that the students will be less concerned with those jobs and salaries which come immediately following graduation, and more concerned with their future after 15 to 20 years in industry.

Many of the present-day dairy leaders are college-trained dairy technologists and, consequently, it is not entirely accurate to infer that our dairy departments have failed entirely in supplying men for industry leadership. Also, progressive dairy departments are continually in the process of revising their curricula and placing emphasis on supporting course work in other areas. Some of the curricula in dairy technology are more encompassing than those in other fields which have been held up as examples by certain members of industry.

If dairy technologists are not assuming key positions in industry, the blame does not lie entirely with the universities. The teachers may be at fault somewhat for failing to encourage the students to obtain proper training—and for not stressing that superior students should not be satisfied with remaining at the lower levels after graduation. However, the teachings and hopes of the educator that his students will set their sights high and strive for professional success will be realized only to the extent that the students have the inherent ability, the necessary desire and the essential pioneer spirit of adventure. Unfortunately, many students lack the initiative, perseverence and the willingness to sacrifice to seek key positions in industry, and are more content to accept that which is immediately expedient than that which over a long run will be more rewarding from the standpoint of professional accomplishment.

Industry, itself, is not blameless for the fact that more trained dairy technologists have not reached key positions in the executive branches. Our industrial leaders have looked to the dairy departments to supply men for the plant production branch of the dairy operation and have been loath to permit good production managers to move into the executive branch. Basically,

a person with a broad training in dairy technology and related subjects and with years of plant production experience, where leadership of men is essential, is well qualified for executive work in a dairy organization. Chemical organizations have found in recent years that many of their better administrators are those who have had technical chemical training and who have risen through the technical branches of the company. The dairy industry can well profit from these practices in other fields.

Dairy plant managers often have inappropriate personnel policies and philosophies in regard to college trained men. These industrialists must recognize that the young men receiving their degrees from the various dairy departments are in the process of development —and desire only an opportunity for continued self-improvement and mental growth. Too often, these young men are used for routine labor for periods of time far exceeding that necessary to give them training in a particular skill. We shall grant that all dairy technology graduates are not qualified to reach the top of the professional ladder, but the least industry can do is to afford the college man with opportunities to utilize his academic training to the best of his natural ability.

In conclusion, I would say that we shall gain nothing by attempting to analyze the causes for the existing personnel situation unless the information obtained serves as a basis for immediate improvement. The shortage of trained man power and low university enrollment are not problems of the dairy field alone, but exist in other branches of science — and competition for superior high school students is keen. Therefore, now is the propitious time for industry and university groups to cooperate in developing mutually acceptable programs which will induce more students to embark upon a career in dairying.

I. A. Gould
The Ohio State University

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JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

W. O. Nelson, Abstract Editor

BOOK REVIEWS

627. Advances in Enzymology, Vol. XIV. Edited by F. F. NORD. Interscience Publishers, Inc., New York, N. Y. 470 pp. $9.25. 1953.

The chapters included are as follows: "Probleme des Energietransports innerhalb lebender Zellen," by Theodor Bücher; "Pantethine and related forms of the *Lactobacillus bulgaricus* factor," by Esmond E. Snell and Gene M. Brown; "Metabolism of phenylalanine and tyrosine," by Aaron Bunsen Lerner; "Oxidation of proteins by tyrosinase and peroxidase," by Irwin W. Sizer; "Chemismus der organischlen Katalyse," by Wolfgang Langenbeck; "Enzymic isomerization and related processes," by Luis F. Leloir; "Suggestions for a more rational classification and nomenclature of enzymes," by O. Hoffmann-Ostenhof; "Quelques techniques nouvelles pour l'etude de la structure des proteines," by Pierre Desnuelle; "Adsorption studies of enzymes and other proteins," by Charles A. Zittle; and "Principles and procedures in the isolation of enzymes," by Sigmund Schwimmer and Arthur B. Pardee. Each chapter is introduced by an outline and terminated by a considerable list of references.

F. E. Nelson

628. Advances in Veterinary Science, Vol. I. Edited by C. A. BRANDLY and E. L. JUNGHERR. Academic Press, Inc., New York, N. Y. 431 pp. $9.00. 1953.

This volume is the first in a planned series designed to provide periodic summaries of the research in the various branches of veterinary medical science. The seven-man advisory board is very international in its membership. All of the contributors to the first volume are working in the United States, but the hope is expressed that future volumes may be more international in character. The chapters are: "Animal diseases and human welfare," by K. F. Meyer; "Virus diseases," by James A. Baker and Charles J. York; "Sulfonamides," by D. K. Detweiler and S. F. Scheidy; "Antibiotics," by S. F. Scheidy and D. K. Detweiler; "The infertility problem of cattle," by David E. Bartlett and Willard S. Boyd; "Bovine mastitis," by W. N. Plastridge; "Swine diseases," by S. H. McNutt; and "Veterinary public health," by J. H. Steele. Each chapter is introduced by an outline of its contents and concludes with a listing of pertinent literature. This book should prove a valuable reference for people in the dairy industry, particularly those engaged in production phases.

F. E. Nelson

BUTTER

629. Effect of a grading program on the concentration of water-insoluble fatty acids in commercial butter. T. R. FREEMAN and J. O. BARKMAN, Dairy Section, Univ. of Ky., Lexington. Am. Dairy Prod. Rev., 15, 7: 2-5. 1953.

Investigations were carried out under practical conditions in Kentucky to determine the effectiveness of the 4-d. grading plan in segregating the types of cream which produce butter with excessive W.I.A. content. Cream was obtained from 15 buying stations during the 4 seasons of the yr. In addition to grading on the basis of age and the 4-d. plan, cream was examined for flavor and odor grade, acidity, sediment, and fat content. Cream from each grade was churned under commercial conditions. W.I.A. analyses were made on the cream after neutralizing and pasteurizing and on the corresponding butter. The study included 27 churnings from 2,654 individual deliveries of cream.

Although the W.I.A. content of cream was generally related to age of the cream, there were definite variations. The correlation was closer in fall and winter than in spring and summer. Organoleptic grade and acidity were not adequate guides to W.I.A. content. Winter cream produced butter with the highest W.I.A. content and fall cream the lowest. No relationship existed between sediment and W.I.A. content or other grading factors. There was wide variation among the churnings in the proportion of W.I.A. in the cream that was carried over into the butter.

T. J. Claydon

Prepared in cooperation with the International Association of Ice Cream Manufacturers and the Milk Industry Foundation.

630. Consumption of butter and margarine in Belgium. A. DIDER, Dept. of Propaganda and Documentation, Office National du Lait et de Ses Derivees. Am. Dairy Prod. Rev., **25**, 6: 2-4. 1953.

Butter consumption in Belgium exceeds that of other food fats. In 1951 consumption increased 22% over prewar years. This increase was greater than that which occurred with other fats. In 1951 the per capita consumption of butter was 23.2 lb. and of margarine 17.21 lb. The increase in butter consumption prevailed even though prices increased relatively more for butter than for margarine, and were 3 to 3½ times as high. Belgians give preference to butter as long as the price is in accordance with the purchasing power of the population. Although there is no special propaganda for butter consumption in contrast to much promotion for margarine, the Belgian consumer has not been induced to lower his consumption of butter in favor of margarine. T. J. Claydon

631. Skunkweed flavor in cream and butter. H. C. OLSON, R. F. BEACHBOARD, R. E. ANDERSON, and L. F. EDMONDSON, Okla. Agr. Expt. Sta., Stillwater. Bull., **B-401**. 1953.

"Skunkweed" is an off flavor found in butter, but not in whole milk or cream. This flavor is in milk taken from cows which have consumed wild carrots and mules tail. An interesting aspect of this problem is that when either weed is consumed separately, the off flavor is either slight or nondetectable, but if they are consumed together, the off flavor is generally very distinct. The corrective measures recommended were complete removal of the cows from infected pastures from May through June (could be earlier or later depending on the season). 2,4-D is known to kill these weeds, but the expense in many cases is not warranted. Removal of cows from infected pastures 2-4 hr. before milking, does not prevent the carry-through of the "skunkweed" flavor into butter made from contaminated milk.

R. W. Hunt

632. Forsoegsmejeriet meddeler: Aeltning af smoer under vacuum (The experiment station reports: Working of butter under vacuum). ANON. Maelkeritidende, **66**, 3: 46-48. 1953.

Working of butter under vacuum of 11-30 cm. mercury in a stainless steel churn resulted in butter which consistently scored higher than the control butter worked at atm. pressure. The consistency of the butter was improved.

A vacuum of 42 cm. mercury proved to be excessive; such butter was often criticized for "grease spots" due to free butteroil. Such high vacuum could be applied, however, without ill effect if it was applied only during the first part and not throughout the entire working procedure, as was done with the lower vacuums.

T. Kristoffersen

633. Over de vissigheid van koelhuisboter (Fishiness in butter on cold-storage). F. E. VAN HAEFTEN and J. W. PETTE. Neth. Milk & Dairy J., 7, 1 & 2: 41-58. 1953.

A discussion of the oxidation defects in dairy products is presented. Winter and summer butter (from ripened cream), both salted and unsalted and with and without the addition of (a) copper, (b) lecithin and (c) copper and lecithin were stored at –18° C. and examined after 3, 5 and 8 mo. Salted butter was less stable than unsalted butter although the difference was much less than expected. The addition of copper produced unstable butter; fishiness, however, developed in summer butter only. Winter butter showed less stability than summer butter, but the flavor defects in the winter butter were less severe. The addition of lecithin did not influence oxidation. Under certain conditions some correlation existed between the organoleptic evaluation of the samples and chemical tests (peroxide number and aldehyde test). Irradiation of butterfat with ultraviolet light did not produce fishiness. Oxidation of pure butterfat at various temperatures did not produce fishiness but a "cod-liver oil" flavor developed. Fishiness appeared to be associated with lecithin and is stimulated in the presence of copper. Summer butter was more susceptible to this defect than winter butter.

W. C. van der Zant

CHEESE

634. Cheddar cheese demands a proper finish. C. SWETT, National Wax Co., Chicago. Butter, Cheese & Milk Prod. J., **44**, 7: 26-27. 1953.

The importance of a proper finish for cheddar cheese in order to maintain its good quality upon lengthy storage is stressed. Bandage wrinkles, high edges and lips are often starting points for mold. The surface of the cheese must be thoroughly dry at the time of waxing.

Some factors to avoid in order to obtain a satisfactory finish are bandage wrinkles, high edges and lips, and moist surfaces at time of waxing.

Blisters are probably the most common cause of finish rejections. These may take the form of gas blisters (raised) or moisture blisters (flat). Pinholes in the wax are often encountered and may be caused by globules of butterfat on the surface, particles of milkstone from dirty hoops, small steam leaks or moisture from other sources in the wax tank. J. J. Janzen

635. Briefing milk — Standardizing calculations for cheese. W. V. PRICE. Butter, Cheese & Milk Prod. J., **44**, 5: 25. 1953.

Suggestions are offered for a simple method of calculating adjustments in standardizing milk for cheddar cheese to meet the standards of fat in the dry matter. Wisconsin Circular 408 offers tables for standardizing milk for cheddar cheese.

A test for casein in milk and nonfat milk (McDowell and Dolby modification of the Walker casein test), and standardizing adjustments involving either fat removal or addition of nonfat fluid or nonfat dry milk are discussed.

An outline of shortened calculations is presented. The results obtained are close approximations of more refined and laborious methods and any inherent errors tend to increase slightly the fat in the dry matter of the finished cheese. Such increases have not been of measurable magnitude. J. J. Janzen

636. Die Reifung von Edamer-Käse in der Verpackung (Ripening of edam cheese after packaging). English summary. E. SCHULZ, G. SYDOW, H. SIEGFRIED, URSULA KOCH and H. POHSE. Die Milchwissenschaft, **8**, 1: 17-22; 2: 52-57; 3: 82-87. 1953.

Edam cheese when ripened in dry cellars develops a very hard rind and loses much weight, whereas if ripened in humid cellars the cheese suffers from surface deterioration produced by molds. Experiments were conducted to remove or minimize both types of defects.

One method which yielded satisfactory results was to paraffin the cheese several days after salting, followed by wrapping with polyamid foil. This method retarded the loss of moisture considerably, but still permitted mold growth, especially if paraffin breakage takes place during ripening and expansion of cheese.

The above method was modified by omitting the paraffining. Edam cheese after pressing and salting was placed for 2 hr. into a 14% special commercial salt solution (J O H A 338) and kept at 14-15° C. The cheese was placed then on sterile wooden shelves to permit the surface to dry (6-8 hr. with one turning). After this the cheese was wrapped with 0.04 mm. thickness polyamid foil and the foil sealed with a hot iron. The wrapped cheese then was exposed to flowing steam for 4-10 min., cooled with cold running water, and transferred to the ripening room kept at 15-17° C. The usual ripening time was 6 wk., during which time the loss in weight amounted to from 0.5 to 1.0%. I. Peters

637. Determination of fat in whey cheese. YNGVE BUCHHOLZ and INGOLF SMITH, Staatl. Norweg. Landwirtschaftlichen Kontrollstation, Oslo. Z. Lebensm.-Untersuch u.-Forsch., 96: 245-248. 1953. (Chem. Abstr., 47: 6569e. 1953.) S. Patton.

638. Cheese and butter knife. P. T. CHAMPLIN. U. S. Patent 2,641,832. 1 claim. June 16, 1953. Official Gaz. U. S. Pat. Office, **671**, 3: 684. 1953.

A small spatula type of knife for slicing cheese and butter, with one side grooved with flutes parallel to the longitudinal direction of the knife and with a series of beads between the flutes. R. Whitaker

639. Cheese press. N. J. PETERS (assignor to Damrow Brothers Co.). U. S. Patent 2,642,797. 1 claim. June 23, 1953. Official Gaz. U. S. Pat. Office, **671**, 4: 1042. 1953.

Details are given for the construction of a horizontal press for pressing cheese in hoops. Pressure is exerted by a ram against the hoops held in place by a back plate designed to be inserted in a pair of a series of holes along the side of the trough. R. Whitaker

CONDENSED AND DRIED MILKS; BY-PRODUCTS

640. De verpakking van volle walsenpoeder in meervoudige papieren zakken (Packing full-cream roller powder in multiwall paper bags). J. C. TH. VAN DEN BERG. Report of the committee for experiments of the Royal Dutch Dairy Federation. 1952.

Full-cream roller powder was stored in 13 different 4-ply paper bags at 20 and 27° C., with a relative humidity of 90 and 65% resp. The pressure on the bags was greater than usually is found in the industry. Fat soaking could be avoided and high quality maintained with a bag constructed with an outer ply of asphalt laminated paper and an inner ply, laminated on the inside with unglazed greaseproof. W. C. van der Zant

641. Problems in packaging nonfat dry milk solids in small packages. M. KLASER. Butter, Cheese & Milk Prod. J., **44**, 5: 27. 1953.

It is recommended that nonfat dry milk solids be so packed as to assure a moisture content of 4% or less at the consumer level, because off flavors develop in the product during storage following an increase of moisture to a level of 5% or more.

The following specific recommendations for the packaging of one pound or smaller containers are made: (1) use only extra grade quality spray nonfat dry milk solids having a moisture content of less than 3%. (2) select a packaging material having the desired physical and chemical characteristics. (3) determine if the package selected will adequately protect the product.

Moisture equilibria determining the % moisture which nonfat dry milk will attain when exposed to a definite percent of relative humidity are as follows: at 10% relative humidity the product will eventually dry to and maintain a moisture of approximately 1.1%; at 20%, 30%, 40% and 50% relative humidity the moisture equilibrium is 2.31%, 3.56%, 5.08% and 5.69% respectively.

The four factors to be considered in selecting packaging materials are: moisture vapor permeability, elasticity range, odor transfer from material, and odor transfer through the material. J. J. Janzen

642. Why quality is important in the packaging of nonfat dry milk. H. SCHWEPPE. Consolidated Badger Co-op., Shawano, Wis. Butter, Cheese & Milk Prod. J., **44**, 6: 19. 1953.

The importance of top quality nonfat dry milk solids when selling for fluid consumption is stressed. The ultimate use of nonfat dry milk in the home presents problems not generally encountered in bulk sales. In order to insure more uniformity and quality every barrel of powder should be graded prior to packaging. The following quality standards have been recommended: butterfat should not exceed 1.25%, moisture content of 3%, preferably lower, lower acidity, solubility of .1 to .05, bacterial estimate 5000 or less, flavor clean and sweet.

The use of a raw milk supply comparable to bottled milk and its proper subsequent handling are of utmost importance for the product to meet with consumer acceptance.

J. J. Janzen

643. Dried milk product for use in coffee or the like and process of producing same. C. A. KEMPF and E. L. BLANCHARD (assignors to Golden State Co., Ltd.). U. S. Patent 2,645,579. 8 claims. July 14, 1953. Official Gaz. U. S. Pat. Office, **672**, 2: 537. 1953.

To prevent dry milk from feathering in coffee, disodium phosphate is added to the milk, prior to condensing, at the rate of 2-3.72%, based on the milk-solids-not-fat, or at the rate of about 1.4-3.1% when the milk has been concentrated to 28-30% total solids, then heating the milk in the range from 205° F. for 15 min. to 320° F. momentarily, before drying.

R. Whitaker

DAIRY BACTERIOLOGY

644. En penicillinresistent syrevaekker. (A penicillin resistant culture). A. J. OVERBY, Roy. Vet. and Agr. Coll., Copenhagen. Maelkeritidende, **66**, 18: 363-366. 1953.

A mixed culture from a cheese-factory was made resistant to up to one unit of penicillin after an adaptation period of one yr. The ability to form flavor was retained. A commercially available mixed culture could not be made resistant when treated in the same manner. The difference was postulated to be due to differences in ability to produce acid of the strains of streptococci in the 2 cultures. The factory culture contained 5 good acid producing strains, while the commercial culture contained 13 strains but only 4 were relatively good acid producers.

Milk containing 0.3 units penicillin per ml., enough to inhibit regular cultures completely, was made into cheese using the factory culture. There was no difference between this cheese and the regular cheese produced in the plant.

T. Kristoffersen

645. A new V-8 medium for determining lactobacilli. F. W. FABIAN, R. D. FULDE and J. E. MERRICK, Dept. Bacteriology and Public Health, Michigan State College, East Lansing. Food Research, **18**, 3: 280-289. 1953.

A new medium containing V-8 juice and brom cresol purple indicator is proposed as an aid in determining lactobacilli and other lactic acid producing organisms. It results in counts comparable to those obtained with other commonly used media but has the advantage of inhibiting many other organisms, while sharply differentiating the lactic acid producers. The latter give rise to colonies which are dark green to black with typical light green to bright yellow halos. Plates must contain fewer than 120 colonies per plate for good differentiation. The method has been used with entire satisfaction in cucumber and kraut fermentation studies.

F. J. Doan

646. Some aspects of the bacteriology of pasteurized milk. IV. The deterioration of laboratory pasteurized milk. TH. E. GALESLOOT. Neth. Milk & Dairy J., **7**, 1 & 2: 1-14. 1953.

Milk was pasteurized in the laboratory for (a) 30 min. at 63° C., (b) 5 min. at 85° C., and (c) 5 min. at 100° C., and was stored at 20 and 27° C. for 24 and 48 hr. Plate counts and bacterial flora were determined. Aerobic bacilli were responsible for the deterioration of the milk upon storage; the predominant defect was sweet curdling. Immediately after pasteurization *B. subtilis* was predominant. Upon storage however, *B. cereus* became predominant in the samples which were pasteurized at 63 and 85° C. The samples pasteurized at 63 and 100° C. showed better keeping quality than those pasteurized at 85° C. According to the author, these results may be attributed to the stimulation of the germination of the spores of *B. cereus* at 85° C. No correlation was found between plate count or number of aerobic bacilli directly after pasteurization and keeping quality of the milk. Incubation at 27° C. proved suitable as an accelerated keeping quality test for pasteurized milk.

W. C. van der Zant

647. Some aspects of the bacteriology of pasteurized milk. V. The deterioration of commercially pasteurized milk (HTST process). TH. E. GALESLOOT. Neth. Milk and Dairy J., **7**, 1 & 2: 15-40. 1953.

Plate counts and bacterial flora of pasteurized milk (HTST) stored at 15, 20 and 27° C. for 24 and 48 hr. were determined. The rate of deterioration of the milk upon storage depended upon the degree of contamination after pasteurization. When little contamination took place, the bacterial flora consisted mainly of *B. cereus* and thermoduric achromobacteria; the main defect then was sweet curdling. In case of a mild or severe contamination defects such as sour *(S. lactis, L. dextranicum)* and various abnormal flavors *(Pseudomonas, Achro-*

mobacter and *Escherichia-Aerobacter* organisms) may develop. The author emphasized the importance of the incubation test at 27° C. as an accelerated keeping quality test for pasteurized milk. W. C. van der Zant

648. Über das Membranfilterverfahren und seine Anwendung als ergänzende hygienische Untersuchungsmethode zur Bestimmung des Coli-Titers in der Milch (Concerning the membrane filter procedure and its use as supplement in the sanitary methods of investigation in determining the coli-titer in milk). English summary. J. Wüstenberg and P. Menke. Die Milchwissenschaft, **8**, 2: 44-48. 1953.

The membrane filter procedure used for the detection of coliform bacteria in water was adopted successfully for the examination of milk.

By means of aseptic technique, small quantities of diluted milk were transferred to a suction filter and deposited on a sterile filter membrane. Suction was applied to remove the excess liquid and the membrane transferred to a petri plate containing solidified Endo agar. After incubating the plate at 37° C. for 18-24 hr., the coliform colonies were readily visible.

This test lends itself to line tests as well as for demonstrations to the working personnel. Methods are described in detail and apparatus is shown. I. Peters

649. Versuche zur Schnellschatzung coliformer Bakterien. (Experiments dealing with rapid estimation methods for coliform bacteria). English summary. J. Borneff. Die Milchwissenschaft, **8**, 2: 48-51. 1953.

A rapid test was developed for the determination of approximate numbers of coliform bacteria in ice cream. To a given volume of ice cream mix was added an equal volume of freshly prepared selective medium containing an aqueous solution 1% peptone, 0.3% KNO_3 (free of nitrites) and 0.015% brilliant green (Note: peptone will decompose brilliant green on standing).

Samples were incubated at 40° C. for 6 hr. Positive tests were obtained under the above described conditions if the original ice cream mix contained 500 or more coliforms / g.

Of 120 commercial samples examined 85% of the samples gave a positive test, indicating a high coliform count.

The present recommended standards for ice cream permit up to 10 coli bacteria / g. Since *Escherichia coli* on the average makes up about 10% of the total coliform count it would mean that the above rapid method would detect all samples having excessive coli counts.

I. Peters

650. Effect of Dahi formation (souring) on the thiamine, riboflavin and nicotinic acid content of milk. R. V. Rao and K. P. Basu, Indian Dairy Research Inst., Bangalore. Indian J. Dairy Sci., **5**, 1: 1-8. 1952.

Of the total milk produced in India, 52.4% is converted into Dahi (curd) either for direct consumption as curd or as an intermediate product in the manufacture of butter. Curds were prepared from boiled milk (cow, buffalo, goat and sheep) with the following cultures: (a) *L. bulgaricus* and *L. dextranicum*, (b) *L. bulgaricus*, (c) *L. acidophilus*, (d) *L. casei*, (e) *S. cremoris* and (f) *S. lactis*. Culture *a* utilized thiamine, riboflavin and nicotinic acid for its growth. After souring, 63.5 and 68.6% of the original thiamine, 76.0 and 78.4% of the original riboflavin, and 82.5 and 79.3% of the original nicotinic acid were retained in the curds of cow and buffalo milk resp. The results obtained with curds from goat and sheep milk were similar. Cultures *b*, *c*, *d*, *e*, and *f* utilized nicotinic acid. *S. cremoris* increased the thiamine content of the curds 18.8 and 10.3% over the amount present in cow and buffalo milk resp.; *S. lactis* to the extent of 6.3 and 5.2%. Riboflavin was destroyed by all cultures except *S. cremoris*. W. C. van der Zant

651. Use of Lactobacillus plantarum 89 as a test organism for assay of riboflavin in dairy products. V. Sreenivasamurthy and K. K. Iya, Indian Dairy Research Inst., Bangalore. Indian J. Dairy Sci., **5**, 2: 53-62. 1952.

L. plantarum 89 was compared with *L. casei* ϵ as a test organism for the assay of riboflavin. It compared favorably with *L. casei* ϵ in giving a linear response, a wide range of sensitivity, and consistent and reproducible results. In addition *L. plantarum* 89 produced a higher total acidity at the assay levels and was less susceptible to the influence of fat and protein in milk. W. C. van der Zant

652. Further studies on the use of L. plantarum 89 for the assay of riboflavin in human, mare and ass milks. V. Sreenivasamurthy and K. K. Iya, Indian Dairy Research Inst., Bangalore. Indian J. Dairy Sci., **6**, 1: 1-8. 1953.

The diluted milks showed an inhibitory effect on *L. plantarum* 89. This effect could be eliminated by precipitation and removal of the proteins. The average values for riboflavin were 0.2 γ/ml., 0.35 γ/ml., and 0.5 γ/ml. for woman's, mare's and ass's milk resp.

W. C. van der Zant

653. Bactericidal efficiency of hydrogen peroxide. III. Use of hydrogen peroxide for preservation of milk. V. K. N. Nambudripad, H. Laxminarayana, and K. K. Iya, Indian Dairy Research Inst., Bangalore. Indian J. Dairy Sci., **5**, 3: 135-146. 1952.

Raw milk with 1,000,000 bacteria or less could be kept in good condition at room temperature (25-28° C.) for 48 to 80 hr., depending on the initial quality, by the addition of 300 p.p.m. of H_2O_2. The keeping quality (clot on

boiling test) of this milk at 30° C. could be increased to 120 to 168 hr. by heating the milk at 63° C. for 30 min. before or after the addition of H_2O_2. Riboflavin, niacin, thiamine, and vitamin B_{12} were not affected by the H_2O_2.
W. C. van der Zant

654. A new method in staining bacteria on microscope slides. P. H. H. Gray. Nature, 171, 4362: 1030. 1953.

Filter paper is soaked in the conventional dyes and cut into small strips after being allowed to dry. When a slide preparation is to be dyed, a drop of distilled water is placed in the fixed film and a strip of dye paper placed on it. Two more drops of water are added to provide sufficient staining solution. When the film is stained, the dye paper is washed off and the film dried. Advantages claimed are: (1) elimination of stains in bottles, (2) economy of dye, (3) economy of storage space, (4) nonspoilage due to oxidation, rubber, etc., (5) ease of preparation, and (6) inexpensive.
R. Whitaker

655. Some studies on microbacteria from Iowa dairy products. S. A. Nashif and F. E. Nelson, Dept. Dairy Industry, Iowa Agr. Expt. Sta., Ames. Applied Microbiol., 1, 1: 47-52. 1953.

Microbacterium lacticum was isolated from 0.01 ml. portions of pasteurized market milk, cheese suspensions, laboratory pasteurized producers milk. One strain was isolated from a dirty milking machine suction hose. The authors conclude that microbacteria are responsible for more than half of the colonies observed on plates from pasteurized products. This group of organisms shows a more marked variability with respect to heat and sugar fermentations than has been reported in the literature.
H. H. Weiser

656. Microbiological assay of vitamin B_{12} with a mutant strain of Escherichia coli. J. S. Chiao and W. H. Peterson, Dept. Biochem., Univ. of Wis., Madison. Applied Microbiol., 1, 1: 42-46. 1953.

A standard curve was reproducible and added vitamin B_{12} was recovered with a mutant strain of *Escherichia coli*. When methionine was present in amounts 50,000 times greater than B_{12} it seriously interfered with the assay. The addition of citrate buffer plus a little bisulfite released more B_{12} from APF samples than water alone.

The mutant test organism, compared to *Lactobacillus leichmannii* (ATCC 4797), gave higher values ranging from 71 to 105% in APF samples and two liver preparations. Several of the samples showed increased B_{12} after cyanide treatment.

Vitamin B_{12} showed greater activity than pseudovitamin B_{12} and vitamin B_{12}. Cyanide apparently had no effect upon these two forms of vitamin B_{12}.
H. H. Weiser

657. The preservation of bacterial cultures under paraffin oil. S. E. Hartsell, Dept. of Biol. Sci., Purdue Univ., Lafayette, Ind. Applied Microbiol., 1, 1: 36-41. 1953.

A variety of bacteria grown in yeast-water agar and veal-infusion agar stabs were successfully preserved for mo. by adding sterile paraffin oil to the surface of the stab. The tubes were stored at 25° C. and transfers made on agar slants of the same medium at various intervals to observe the viability and physiological characters of the test organisms. No apparent physiological changes were noted during storage.
H. H. Weiser

658. Antibiotics and lactic acid starter cultures. E. I. Stoltz and D. J. Hankinson, Dept. of Dairy Industry, Univ. of Mass., Amherst. Applied Microbiol., 1, 1: 24-29. 1953.

Hansen's lactic ferment culture was propagated in sterilized antibiotic-free skimmilk. Effects on bacterial growth, changes in pH and titratable acidity were observed when 1.0 or 0.1 unit of penicillin; 5.0, 1.0 or 0.1 mg. of streptomycin; 5.0 mg. of streptomycin and 1.0 unit of penicillin in combination, 1.0 mg. streptomycin and 0.1 unit of penicillin in combination, 0.1 mg. streptomycin and 0.01 unit of penicillin, 0.50, or 0.10 mg. of aureomycin; and 5.0 or 1.0 mg. tyrothricin were added.

The authors conclude that milk containing these concentrations of antibiotics should not be used in the processing of cultured dairy products. The addition of 75,000 units of penicillin to a milk supply would appreciably inhibit the production of lactic acid in 200 gallons of milk.
H. H. Weiser

659. Effect of residual penicillin in milk on the dye-reduction tests for quality. C. K. Johns and J. G. Desmarais, Dept. Agr., Ottawa. Rev. can. biol., 11: 512. 1953. (Chem. Abstr., 47: 6567f. 1953.)
S. Patton

660. Survey of coliform organisms in ice cream. Leon Buchbinder, Walter C. Bartsch, W. A. Cordes, and Leo Habel, New York City Dept. of Health. Am. J. Public Health Yearbook, 76-83. 1953. (Chem. Abstr., 47: 6568d. 1953.)
S. Patton

661. The reactions of Escherichia coli to penicillin. Kyozo Masuda, Kyoto Univ. Med. Coll. Arch. Antibiotics (Japan), 3: 100-119. 1950. (Chem. Abstr., 47: 5989b. 1953.)
S. Patton

662. Microbiological detection and titration of antibiotics in milk and milk products. J. Jacquet and L. Steeg, Fac. sci., Caen, France. Ann. fals. et fraudes, 46: 5-14. 1953. (Chem. Abstr., 47: 5996f. 1953.)
S. Patton

DAIRY CHEMISTRY

663. A comparison of the Schain butterfat test with the Babcock method. W. H. Kanninen and H. Lawrence, Foster D. Snell, Inc., New York, N. Y. Milk Dealer, **42**, 9: 48-50. 1953.

Data are presented to show that the results obtained by the Schain method are statistically closer to those of the ether extraction methods than is the case with the Babcock test. The comparative precision of the two methods referred to the control method is of the order of .089% average deviation for the Babcock test as compared to .056% for the Schain test.

The advantages of the Schain method are listed. Because of the advantages, accuracy and convenience of the method, the Schain test is particularly well suited for field testing as well as laboratory use. It was concluded that the Schain method is a solution to the problem of a butterfat test which requires no expensive equipment or dangerous chemicals, is easy to perform in the field by the average milk producer, and which is at least as accurate as the Babcock test. C. J. Babcock

664. Dairy products improved by ion exchange. O. F. Garrett, M. & R. Dietetic Laboratories Inc., Columbus, Ohio. Food Eng., **25**, 7: 61. 1953.

By using the newer resinous ion exchange materials it is possible to produce such products as stabilized evaporated milk, lactose, special milk powders, and quickly dispersable cream powders. In the process, the cation exchanger may be used either alone or in sequential combination with an anion exchanger to substitute mineral elements or give a product that is almost completely demineralized.

T. J. Claydon

665. Method of producing thyroprotein. E. S. West and J. T. Van Bruggen (assignors to Feed Products Co.). U. S. Patent 2,642,426. 4 claims. June 16, 1953. Official Gaz. U. S. Pat. Office, **671**, 3: 850. 1953.

A method for making thyroprotein suitable for animal feed by adding to a 5-7.5% casein solution at 150-175° F. adjusted to pH 8-9 with $NaHCO_3$, $KMnO_4$ as a catalyst at the rate of 1-4 oz. per 300 lb. casein, then adding I at the rate of 45-72 lb. per 300 lb. of protein over a period of 15-30 min., while continuously sparging the solution with O_2. After the addition of the I, the temp. is raised to 180-190° F. and the sparging continued until the desired thyroprotein content is obtained. The thyroprotein may be prepared in dry form from the soln. by acidifying to coagulate it, and drying and grinding the ppt. R. Whitaker

666. Quantitative estimation of the total solids in fresh whole milk by oxidimetry. A. G. Leggatt. Nature, **171**, 4362: 1028. 1953.

Ten ml. of milk is diluted to 100 ml. with distilled water and well mixed. Transfer 10 ml. of the diluted milk to a white cup containing 20 ml. of distilled water. Add 2 drops of ferrous-o-phenanthroline. Continuously titrate against .01 N ceric sulphate in N H_2SO_4 until colorless. Continue titrating after a lapse of 1 min. The author recognizes that the method in its present form may not be a practical test for total solids in milk, but believes a rapid and accurate test may be developed based on oxidimetry. R. Whitaker

667. Standardization of methods for the analysis of milk and dairy products in the Netherlands. V. The determination of the density of milk at 20° C. with a lactodensimeter and the calculation of the percentage of total solids in milk. The scientific committee for the standardization of methods for the analysis of milk and dairy products. Neth. Milk & Dairy J., **7**, 1 & 2: 90-98. 1953.

A detailed description of the lactodensimeter and procedure is presented. Prior to the determination, the milk is held at 40° C. for 5 min. and then cooled to 20° C. The following formula is used in the calculation of the total solids content: $D = 1.23V + 2.6 \times 100(d^{20} - 0.9982)/d^{20}$, in which D = the calculated percentage (by weight) of total solids, V = the percentage (by weight) of fat, and d^{20} = the density of the liquid (in g./ml. at 20° C.). In many cases, especially in case of whole milk of normal fat content (3 to 5%) the following formula may be used: $D = (5V + g)/4 + 0.45$, in which g is the number of "densimeter degrees" of the reading at 20° C.

W. C. van der Zant

668. Studies in physico-chemical properties of milk. II. A simple method of estimating lactose content of milk by oxidation with $KMNO_4$. B. R. Puri and P. Singh, Dept. of Chem., Punjab Univ., Hoshiarpur. Indian J. Dairy Sci., **5**, 1: 39-44. 1952.

A simple method is described for the routine analysis of lactose in milk, based on its oxidation by acidified $KMNO_4$.

W. C. van der Zant

669. Studies in physico-chemical properties of milk. III. Determination of size distribution of fat globules in milk by the application of Stokes' law. B. R. Puri, M. L. Lakhanpal, and S. C. Gupta, Dept. of Chem., Punjab Univ., Hoshiarpur. Indian J. Dairy Sci., **5**, 4: 189-199. 1952.

The rise of fat globules in milk and globule size distribution was determined. A micropipette arrangement was used for the fractionation of the fat globules. The average globule diameter for buffalo, cow, and goat milk was 4.05-4.8 μ, 3.59-4.0 μ, and 2.88-3.42 μ resp. Globule size distribution curves showed maxima at 3.75 or 4.25 μ, 3.75 μ, and 0.25 and 2.75 μ for buffalo,

cow, and goat resp. The globule size appeared to be independent of the fat content of the milk, other factors being the same.
W. C. van der Zant

670. Accuracy of formol titration in estimating total and casein nitrogen contents of milk. A. M. El-Sokkary and H. A. Hassan, Ibrahim Pacha Univ., Shebin El-Kome. Indian J. Dairy Sci., **6**, 1: 37-39. 1953.

The total and casein nitrogen content of milk (cow and buffalo) was determined by the Kjeldahl method and the formol titration. The results obtained by both methods agreed well, provided that proper conversion factors were used to calculate the total and casein nitrogen content. W. C. van der Zant

671. Studies on the refractive index of milk. C. V. Ramakrishnan and B. N. Banerjee, Dept. of Biochem., Indian Inst. of Sci., Bangalore. Indian J. Dairy Sci., **5**, 1: 25-31. 1952.

The effect of locality, season, feed, acidity and storage, processing, addition of water, skimmilk, and sugar and water on the density, refractive index, and refractive constant of milk (cow and buffalo) was studied. Winter milk gave higher values for refractive index than samples collected in other mo. The density and refractive index were higher when cottonseed cake was fed than with grass only. Addition of water or skimmilk, and sugar to watered milk lowered the refractive index. The density and refractive index of prepartum milk decreased; these values were irregular during the colostrum period but returned to normal within a wk. after calving. W. C. van der Zant

672. Cholesterol content of milk of cows and buffaloes. S. L. Gulvady, A. Kannan, and K. P. Basu, Indian Dairy Research Inst., Bangalore. Indian J. Dairy Sci., **5**, 3: 125-134. 1952.

Samples of bovine milk and buffalo milk had a cholesterol content of 1-5 mg. and 1-8 mg. per 100 ml. resp. A gradual decrease in the cholesterol content was observed as lactation advanced. The cholesterol content increased at the end of the lactation period.
W. C. van der Zant

673. Non-protein nitrogenous constituents of milk. I. Variation due to species, breed, individuality, season and stage of lactation. D. Venkatappaiah and K. P. Basu, Indian Dairy Research Inst., Bangalore. Indian J. Dairy Sci., **5**, 2: 95-116. 1952.

N.P.N. (ammonia N, amino acid N, urea N, uric acid, creatinine and creatine) was determined in milk of buffaloes, goats, sheep, and cows of different breeds. The percentage of N.P.N. to total N was approx. the same in milk of different species and breed. Little difference was found in the N.P.N. constituents of milk of different breeds of cows. Compared with cow's milk, sheep's milk had a high N.P.N. and amino acid N content; goat's milk had a high urea content. With the exception of creatine, which was constant, all N.P.N. constituents were higher in the colostrum of cow and buffalo than in normal milk. W. C. van der Zant

674. Casein heterogeneity and rennet coagulation. G. T. Pyne. Chemistry & Industry, 171-172. 1951. (Chem. Abstr., 47: 6450i. 1953.) S. Patton

675. Reaction between proteins and reducing sugars in the dry state. V. Reactions of D-galactose, 2-deoxy-D-galactose, D-glucosamine, and N-acetyl-D-glucosamine with casein. C. H. Lea and D. N. Rhodes, Univ. Cambridge, Engl. Biochim. et Biophys. Acta, 9: 56-60. 1952. (in English). Chem. Abstr., **47**: 6452d. 1953.) S. Patton

676. Electrophoretic studies on enzymically modified ovalbumin and casein. Gertrude E. Perlman, Rockefeller Inst. Med. Research, New York, N. Y. — Discussions Faraday Soc., 13: 67-77. 1953. (Chem. Abstr., 47: 6472b. 1953.) S. Patton

677. The isolation of phosphothreonine from bovine casein. Carl Henric de Verdier, Univ. Uppsala, Sweden. Acta Chem. Scand., 7: 196-200. 1953. (in English). (Chem. Abstr., **47**: 5971b. 1953.) S. Patton

678. Effect of ultraviolet radiation upon the citric acid present in milk. W. Diemair and G. Manderscheid-Schwinding, Univ. Inst. Lebensmittelchemie, Frankfort a. M. Main, Ger. Z. Anal. Chem., 138: 179-186. 1953. (Chem. Abstr., 47: 6062c. 1953.) S. Patton

679. Fluorescein-conjugated bovine albumin. Physical and biological properties. Alfred A. Schiller, Richard W. Schayer, and E. L. Hess, Univ. of Illinois Coll. of Med., Chicago. J. Gen. Physiol., **36**: 489-506. 1953. (Chem. Abstr., 47: 6016e. 1953.) S. Patton

DAIRY ENGINEERING

680. Forced draft vacuum stack for milk driers. G. E. Shaw. U. S. Patent 2,644,391. 1 claim. July 7, 1953. Official Gaz. U. S. Pat. Office, **672**, 1: 132. 1953.

A stack or chimney arrangement for exhausting the vapors from roll driers to the outside atmosphere above the roof. Feed pipes from each drier funnel into one large vertical stack. Inside each feed pipe is a smaller pipe or air duct, which terminates part way up the stack. Blowers in the ducts and a system of baffles in the stack, provide a forced draft up the stack.
R. Whitaker

681. Sanitary pump. D. F. Thomas (assignor to Waterous Co.). U. S. Patent 2,642,808. 9

claims. June 23, 1953. Official Gaz. U. S. Pat. Office, **671**, 4: 1046. 1953.

The construction of the two impellers of a sanitary pump of the positive action type is described in detail. R. Whitaker

682. Valve construction. E. N. BURNETT (assignor to Gerber Products Co.). U. S. Patent 2,642,894. 2 claims. June 23, 1953. Official Gaz. U. S. Pat. Office, **671**, 4: 1070. 1953.

A sanitary valve of the plug type, held in place by a spring but easily turned by lifting slightly on the handle. R. Whitaker

683. Pasteurizing methods and apparatus. J. A. CROSS (assignor to Mojonnier Bros. Co., Inc.). U. S. Patent 2,644,758. 7 claims. July 7, 1953. Official Gaz. U. S. Pat. Office, **672**, 1: 235. 1953.

Details are given for the construction and operation of a pasteurizer and cooler, where cooling is accomplished by spraying the hot pasteurized product into a vacuum, and the vapors produced are superheated and utilized to provide the medium for indirectly heating the raw milk. R. Whitaker

DAIRY PLANT MANAGEMENT AND ECONOMICS

684. Case-handling hurdles cleared by automation. J. SHINN, McGraw-Hill News Bureau, Los Angeles. Food Eng., **25**, 7: 67-70. 1953.

At the plant of Arden Farms Co., Los Angeles, installation of automatic case handling equipment has streamlined operations and saved $40,000 yearly on labor. The new system includes 3 case combiners, 2 case stackers, 2 elevators, and conveyors. Cases from the filling machines are grouped and stacked in lots of 5. Stacks are conveyed to refrigerator storage and/or loading docks. Operation details of the combiners and stackers are given.
T. J. Claydon

685. Insulated bags and insulated packages. ANON. Sou. Dairy Prod. J., **54**, 1: 130-131. 1953.

Among 1,000 housewives who have had experience with both types of insulated containers, 44% preferred insulated bags, 21% insulated packages, 4% plain packages and 30% had no preference, according to a survey conducted for the Borden Co. F. W. Bennett

686. Carnation OK's LPG. ANON. Food Eng., **25**, 6: 56-57. 1953.

LPG (liquefied petroleum gas) has been used in motor vehicles of Carnation Co., Los Angeles for over 13 years. The cost of converting a truck for LPG averages about $225. When a fleet of trucks is operated with the new gas, dispensing facilities for fuel are necessary. Installation of a 6000 gallon LPG tank costs approx. $3,500. Before converting, consideration should be given to zoning regulations, type of gas to be used, motor overhaul, and storage tank installation. T. J. Claydon

687. We are helping ourselves. ANON. Butter, Cheese & Milk Prod. J., **44**, 7: 28-29. 1953.

The American Dairy Association reports butter consumption is taking an upward turn. Figures released for Apr. show an increase of 14,132,000 lb. of butter over the corresponding mo. in 1952. The basic reasons for the consumption increase advanced are a slight decline in price, renewed promotional efforts, and an overall increase in living standards.

Methods used by the American Dairy Association in promoting increased consumption include colorful nation-wide advertising, publicizing dairy reducing diets, and encouraging restaurants and hotels to provide two pats of butter with a meal. J. J. Janzen

FEEDS AND FEEDING

688. Dried skimmilk in rations for dairy calves. R. E. LEIGHTON and J. S. HUFF, Texas Agr. Expt. Sta., College Station. Progress Rept. 1482. 1952.

One lb. of dried skimmilk mixed with one gal. of warm H_2O, given in 2 feedings per d., is a satisfactory replacement for each gal. of whole milk in rations of dairy calves 3 wk. old and older. R. W. Hunt

689. Value of early hay cutting is shown by tests. ANON., Miss. Agr. Expt. Sta., State College. Miss. Farm Research, **16**, 3: 1. 1953.

Results of 2 yr. work show that although total dry matter produced per acre from Johnson grass hay is higher when the crop is cut in the late milk stage, the lb. of wt. gained in dairy heifers per acre of hay is considerably higher when the crop is cut in the early boot stage. R. W. Hunt

690. Undersoegelser af ensilage og goedningsproever fra ensilagefodrede koer (Analysis of silage and manure samples from silage fed cows). English summary. C. LIND, State Expt. Sta. Creamery, Hilleroed, Denmark. Bull. 78. 1953.

Beet top silage conserved with AIV acid generally was of good quality when pH initially was brought down to 3.15-4.1. As the pH increased above 4.1 the quality became increasingly poorer. Butyric and isovaleric acid, NH_3-N and the numbers of anaerobic spore-forming bacteria were used as criteria for quality.

Evaluation of silage by appearance, odor and taste did not always correspond to the chemical evaluation.

Good silage of pH 4.1 and below usually resulted in manure with a relatively low con-

tent of anaerobic sporeformers, 10^4 or less per gram. Manure with counts beyond 10^6 per gram could result from silage of pH 4.25 or above.
T. Kristoffersen.

691. Roughage quality and milk production. G. W. TRIMBERGER, Cornell Univ., Ithaca, N. Y. Guernsey Breeders' J., **88**, 5: 659-660. 1953.

Different methods of harvesting and storing mixed legume and timothy roughages were compared. When harvested at the same time, no important differences in feeding value were obtained as barn dried hay, grass silage or field cured hay. However, the harvesting and storage losses were 15, 21, and 24%, respectively.

The ave. dry matter retained for feeding for a two-year ave. was: early-cut, barn-dried hay 84.5%, early-cut grass silage 79.3%, late-cut, field-cured hay 77.5%. The digestible dry matter at the different times of cutting was approximately 70% for early-cut grass silage, 60% for early-cut, barn-dried hay and 50% for late-cut, field-cured hay.

All of these roughages contained sufficient carotene to meet the requirements of most milking cows, but the carotene content of the early-cut grass silage was enough higher than the others to significantly increase the carotene and vitamin A content of the blood plasma and of the milk produced by the cows to which it was fed. A. R. Porter

692. Improving straws by alkali, water, or lime treatment. N. D. KEHAR, Indian Vet. Research Inst., Izatnagar. Indian Farming, **2**, 11: 14-17, 23. 1953. (Chem. Abstr., 47: 6573b. 1953.) S. Patton

693. Comparative feed value — sweet corn, pea-vine, field-corn silage. GUSTAV BOHSTEDT, Univ. of Wis., Madison. Canning Trade, **75**, 41: 7. 1953. (Chem. Abstr., 47: 6573e. 1953.) S. Patton

694. Tocopherol content of farm feeds. FRED BROWN, Hannah Dairy Research Inst., Kirkhill, Ayr, Scot. J. Sci. Food Agr., **4**, 161-165. 1953. (Chem. Abstr., 47: 6067b. 1953.) S. Patton

695. Simple and rapid determination of silica in cattle feeds. W. A. C. CAMPEN, Rijkslandbouwproefsta., Maastricht, Netherlands. Chem. Weekblad, 48: 579. 1952. (Chem. Abstr., 47: 6067e. 1953.) S. Patton

696. Zinc content of Kansas grown alfalfa. W. G. SCHRENK and J. E. HAWLEY, Kansas Agr. Expt. Sta., Manhattan. Trans. Kansas Acad. Sci., 55: 489-492. 1952. (Chem. Abstr., 47: 6067f. 1953.) S. Patton

GENETICS AND BREEDING

697. Forsoeg med tilsaetning af penicillin og streptomycin til fortyndingsvaedsken (Experiments with addition of penicillin and streptomycin to the diluent). K. ROTTENSTEN and P. S. OESTERGAARD, Roy. Vet. and Agr. Coll., Copenhagen. Expt. Laboratory Bull. 265. 1953.

From 1949-51 experiments were conducted to establish the effect of addition of antibiotics to diluent used with semen. The diluent consisted of 2.6 g. Na citrate, 1.0 g. glucose, 0.3 g. sulfanilamide, 3.5 g. gelatine and 100 g. of dist. H_2O. The semen was diluted 5-15 times. Before dilution 20% eggyolk was added. One-half ml. of diluted semen was used for insemination.

Addition of 400 units per ml. of the potassium salt of G-penicillin improved the rate of pregnancy from 49.8 to 51.3%. 400 units of penicillin plus 1000 units of streptomycin (dihydrostreptomycin sulfate) increased the rate of pregnancy from 53.0 to 54.1% over penicillin alone. 500 units of streptomycin alone was superior to 400 units of penicillin; the rate of pregnancy was 49.6% with no addition, 51.6% with penicillin and 54.6% with streptomycin.

Penicillin improved the fertility of fertile bulls 1.0% and the fertility of infertile bulls 8.1%. For streptomycin the corresponding figures were 2.6% and 12.9%. However, the fertility levels of infertile bulls never quite reached that of fertile bulls.

In the experiment 15 bulls were used and about 120,000 inseminations performed.
T. Kristoffersen

698. Animal pregnancy tests studied. ANON., R. I. Agr. Expt. Sta., Kingston. R. I. Research Quart. Rev., 45. 1953.

A report recently made from the Bellevue Medical Center concerns the use of secretions of the cervex to determine pregnancy in humans. Work is underway to determine the adaptability of this test to dairy cattle. The technique now being used at the Univ. of R. I. consists of collecting a mucus sample from the mouth of the cervex, allowing it to dry on a slide, and examining it with a microscope. One pattern indicates pregnancy and another indicates the animal is "open." At this stage, the % of inaccuracy is high. However, many of the causes are being rectified. R. W. Hunt

699. Motility of ram and bull spermatozoa in dilute suspension. A. W. BLACKSHAW, Univ. Sydney, Australia. J. Gen. Physiol., 36: 449-462. 1953. (Chem. Abstr., 47: 6016c. 1953.) S. Patton

700. Cell genetics and hereditary symbiosis. J. LEDERBERG, Dept. of Genetics, Univ. of Wis., Madison. Physiol. Rev., **32**, 4: 403-430. 1952.

The reviewer reported that controversies regarding the interpretation and scope of cyto-

plasmic inheritance often center on the diagnosis of virus vs. plasmogene. The objective of the review was to reconcile these views while looking for further implications in the synthesis. 227 references were cited. E. G. Moody

HERD MANAGEMENT

701. Dehorning device. A. L. Otis. U. S. Patent 2,645,011. 5 claims. July 14, 1953. Official Gaz. U. S. Pat. Office, **672**, 2: 376. 1953.

A cattle dehorning tool consisting of a circular saw and a horn hook, which guides the horn against the saw. R. Whitaker

702. Milk cooler. V. A. Haines (assignor to International Harvester Co.). U. S. Patent 2,644,604. 6 claims. July 7, 1953. Official Gaz. U. S. Pat. Office, **672**, 1: 192. 1953.

A cabinet for cooling milk in cans, having rods across the cabinet with U shaped brackets which hold the cans in place and submerged to the neck of the cans in the cooling medium. R. Whitaker

703. Dehorning device. W. L. Kuhns. U. S. Patent 2,643,454. 1 claim. June 30, 1953. Official Gaz. U. S. Pat. Office, **671**, 5: 1299. 1953.

A device is described for dehorning cattle and cutting recesses in the bony ridge at the base of horns. It consists of two blades with handles attached and pivoted together. R. Whitaker

704. Cowtail suspender. S. Jaksekovich. U. S. Patent 2,645,203. 8 claims. July 14, 1953. Official Gaz. U. S. Pat. Office, **672**, 2: 431. 1953.

This device, attached to the ceiling over the rear end of the cow, clips to the cow's tail and holds it securely during milking. R Whitaker

705. Cow stall structure. H. J. Merrill. U. S. Patent 2,642,037. 4 claims. June 16, 1953. Official Gaz. U. S. Pat. Office, **671**, 3: 744. 1953.

A design for a stall or manger, the dividing structure being constructed of pipe and fittings imbedded in part in the concrete floor material. R. Whitaker

706. Stock restraining gate. A. S. Hill and R. H. Hill. U. S. Patent 2,642,840. 7 claims. June 23, 1953. Official Gaz. U. S. Pat. Office, **671**, 4: 1055. 1953.

A device for holding farm animals in a rigid position, consisting of a frame and two parallel jaws which move together as the result of manipulating a pivoted lever. R. Whitaker

707. Automatic drinking trough. G. Marechal. U. S. Patent 2,642,839. 1 claim. June 23, 1953. Official Gaz. U. S. Pat. Office, **671**, 4: 1055. 1953.

A vertical plate, pivoted on top, when pressed by the nose of an animal opens a spring loaded valve releasing water into a drinking fountain bowl or trough for stock. R. Whitaker

708. A Kymograph. W. H. M. Morris, Cornell Univ., Ithaca, N. Y. Agr. Eng., **34**, 6: 412. 1953.

A kymograph for recording time studies of dairying operations is described and illustrated.

Requirements for the time and motion study included ability of one observer to record accurately the actions of one or two operators, possible irregular cycles of operations, little attention required for recording by observer, observation and recording to be achieved with minimum of fatigue.

The recording instrument uses an adding machine carriage, a synchronous motor for driving the paper roll, a clutch, and a symbol recording device. Elements of 0.01 min. duration can be recorded. H. L. Mitten, Jr.

ICE CREAM

709. Vegetable oils and ice cream. W. A. Wentworth, Borden Co., New York. Sou. Dairy Prod. J., **54**, 1: 81-83. 1953.

On the basis of sales data for the last 7 mo. of 1952 from plants in 7 midwestern cities, the production and sale of vegetable oil frozen desserts had no significant effect upon the utilization of butterfat in the plants where they were produced but it helped to increase the use of serum solids by selling them to those who were not frozen dessert consumers before. F. W. Bennett

710. A final report on shrinkage research. D. R. Frazeur and C. D. Dahle, Pa. State Coll., State College. Ice Cream Rev., **36**, 12: 87. 1953.

The authors present a concise and informative summary of ice cream shrinkage. The report covers the effects of proteins, enzymes, ingredients and mechanical factors on shrinkage. W. J. Caulfield

711. The significance of coliforms in ice cream. H. C. Olson, Okla. A. and M. Coll., Stillwater. Ice Cream Rev., **36**, 12: 114-118. 1953.

High coliform counts in ice cream are indicative of a lack of sanitary measures. Among the common causes for high coliform counts are the addition of any raw dairy product to ice cream mix, use of faulty equipment which may result in improper pasteurization, storage of mix too long and at too high a temperature, and contamination from personnel, improperly cleaned and sterilized equipment, and contaminated ingredients. Measures are suggested for the control of coliform bacteria in ice cream. W. J. Caulfield

712. Soft ice cream freezers. C. B. WELLS, JR. General Equip. Sales, Inc., Indianapolis. Southern Dairy Prod., J. **53**, 5: 92-94. 1953.

The construction requirements of freezers vary with the composition of the mix. Compressors for "soft ice cream" freezers usually are not separated from the freezing room but they may be more efficient and the room kept cooler by having them outside. One type freezer is the small batch type machine in which the product must not be frozen too much to allow removal of the product by simple dumping. A second type is one in which the mix is forced continuously through the barrel by a pressure pump. The third type is a simple auger fed freezer. The two latter types will deliver a firmer product than the first. The capacity of the freezer must not be exceeded in order to avoid delivery at a higher temperature than is desired. F. W. Bennett

713. Composition control of ice cream. J. A. MEISER, P. S. LUCAS, and J. W. KEARNEY, JR., Mich. Agr. Expt. Sta., East Lansing. Sou. Dairy Prod. J., **53**, 5: 164-167. 1953.

Lack of uniform composition of ice cream may result in monetary losses due to fines or confiscation, excessive amounts of ingredients used and lower consumer acceptance. Inadequate knowledge of the composition of ingredients, errors in mix calculations, measuring by volume instead of weight, spillage, incomplete dumping, failure to check and standardize mixes, lack of sufficient agitation of mixes, canning mix directly from coolers, rising of fat even in homogenized mix, insufficient agitation of mix and addition of flavoring and coloring materials were each shown to cause variations. Proper equipment, competent laboratory personnel, and management placing the proper responsibilities upon each department are essential to controlling variations. F. W. Bennett

714. Coliforms in ice cream require cautious interpretation. W. C. BARTSCH, Borden Co., New York. Sou. Dairy Prod. J., **54**, 1: 51. 1953.

In the Borden plant the logarithmic average bacteria count of 166 samples of ice cream was 800 per g. The mix was pasteurized at 165° F. for 30 min. and periodic phosphatase tests were always negative. In spite of efforts to minimize foam, 4 to 6 in. of foam floated on the surface of the mix at the close of pasteurization. The incidence of coliform bacteria in the foam was 1 or more per g. in 8.55% and 10 or more per g. in 1.97% of the samples. In ice cream samples taken from the plants and stores, 58.3% contained 1 or more per g. and 30.4% contained 10 or more coliforms per g. Survival of pasteurization in the foam, in addition to possible contamination, should be considered as a possible source of coliforms in ice cream. F. W. Bennett

715. Composition of low fat frozen desserts. P. H. TRACY, Univ. of Ill., Urbana. Ice Cream Rev., **36**, 12: 110. 1953.

Ice milks are sold either soft direct from the freezer or in the hardened state. The soft served product should be stiff and cold when served with particular emphasis upon its refreshing quality. In the hardened product smoothness and chewiness are characteristics which are desirable. Because of these differences, it is not possible to achieve the desired characteristics in both types of product from the same mix.

Composition standards are suggested for each type of ice milk. In the opinion of the author many manufacturers have produced a soft product which is too sweet, too high in serum solids and corn syrup solids to be refreshing. W. J. Caulfield

716. Apparatus for removing frozen confections from spurs. H. G. HUEBSCH. U. S. Patent 2,644,409. 16 claims. July 7, 1953. Official Gaz. U. S. Pat. Office **672**, 2: 137. 1953.

To facilitate removal of frozen confections from spurs, a resistance wire imbedded in each warms the spur when a small electric current is applied. R. Whitaker

717. Production of ice cream blocks. R. TURNER. U. S. Patent 2,643,526. 7 claims. June 30, 1953. Official Gaz. U. S. Pat. Office, **671**, 5: 1319. 1953.

Stiff ice cream is extruded onto a flat conveyor belt which passes through a freezing tunnel and is delivered to a cutting machine as a strip of hardened ice cream ready for cutting into portions suitable for packaging. R. Whitaker

718. Means for making ice cream. M. A. ORFITELLI. U. S. Patent 2,642,009. 1 claim. June 16, 1953. Official Gaz. U. S. Pat. Office, **671**, 3: 736. 1953.

A refrigerator tray with a small sliding agitator for stirring homemade ice cream as it is frozen. R. Whitaker

719. Stick dispensing machine. R. F. ANDERSON. U. S. Patent 2,645,398. 13 claims. July 14, 1953. Official Gaz. U. S. Pat. Office, **672**, 2: 486. 1953.

A device for inserting wooden sticks in a stick holder for making frozen confections in a brine tank. R. Whitaker

720. Method of making sandwiches. C. W. GENTRY. U. S. Patent 2,642,822. 2 claims. June 23, 1953. Official Gaz. U. S. Pat. Office, **671**, 4: 1050. 1953.

Ice cream sandwiches are made by slicing open end containers of hard ice cream to form the sandwich filling material and inserting the slices between two cakes or wafers to form a

sandwich protected by the sliced container surrounding the filling. R. Whitaker

721. Method of making cake center frozen confections in twin molds. C. O. LUND (assignor to Maun & Lund, Inc.). U. S. Patent 2,642,820. 6 claims. June 23, 1953. Official Gaz. U. S. Pat. Office, **671**, 4: 1049. 1953.

A thin piece of cake, coated with a layer of vegetable oil, is inserted in the center of a twin "pop" mold containing sufficient mix so that the mold is full and the cake is fully submerged. The product is then frozen in the usual manner in a brine tank. R. Whitaker

722. Ice cream cone dipping apparatus. A. D. CAMPBELL. U. S. Patent 2,642,029. 2 claims. June 16, 1953. Official Gaz U. S. Pat. Office, **671**, 3: 742. 1953.

A basket, with pockets for holding cones filled with ice cream in an inverted position, rests on a tray like support which slides up and down on two upright rods. When in the lowered position, the top of the ice cream dips into a bath of coating material; when raised the cone carrying basket may be removed after draining. R. Whitaker

MILK AND CREAM

723. Thermal effectiveness of high-temperature, short-time pasteurization. J. L. FOLEY and D. J. HANKINSON, Mass. Agr. Expt. Sta., Amherst. Bull. 462, 42-43. 1953.

Ten commercial HTST milk pasteurizers were evaluated. Thermocouples were inserted between the plates in the heating section and pasteurizer regeneration section to obtain temperature readings. These readings then were used to determine phosphatase lethality curves. The pasteurizers studied contributed 213-390% of the heat necessary to inactivate the enzyme, while the heating and cooling portions contributed 20-108% of the heat needed. R. W. Hunt

724. A rational approach to fluoridation (using milk). JACK E. MCKEE, Calif. Inst. of Technol., Pasadena. J. Am. Water Works Assoc., 45: 376-386. 1953. (Chem. Abstr., 47: 6583a. 1953.) S. Patton

725. Chocolate dairy beverage powders. MORRIS B. JACOBS. Am. Perfumer Essent. Oil Rev., 60: 291-292. 1952. (Chem. Abstr., 47: 6066e. 1953.) S. Patton

726. The optimum vehicle for fluoridation — water or milk. J. E. MCKEE, Calif. Inst. of Technol., Pasadena. J. New Engl. Water Works Assoc., 67: 9-29. 1953. (Chem. Abstr., 47: 6582i. 1953.) S. Patton

727. Refrigerated retail delivery trucks for milk and ice cream. ANON. Milk Dealer, **42**, 9: 45. 1953.

Meyer Sanitary Milk Co. of Kansas City, Kansas is operating the nation's first entirely refrigerated fleet of dairy trucks, on both wholesale and retail delivery routes, designed to carry not only milk and by-products, but also ice cream. The retail trucks are of the stand-drive type with a special low temperature compartment for ice cream and other refrigerated compartments for milk and by-products. The trucks are refrigerated while in the garage at night by means of hold-over plates containing an eutectic solution which is chilled by the plant's ammonia refrigeration system. C. J. Babcock

728. Pricing milk on solids content — Different prices paid to producers for milk are based on percentage of fat and nonfat solids. D. A. CLARKE, JR. and J. B. HASSLER, Univ. of Calif., Berkeley. Calif. Agr., **7**, 1. 1953.

A practical and accurate test for fat exists, but there is no economically feasible method of testing for nonfat solids under plant conditions. However, dairy chemists have established the existence of a reasonably reliable relationship between amounts of fat and nonfat solids in whole milk. This relationship can be used to estimate the nonfat solids content of milk. Two methods of determining the price of milk based on a nonfat solids content are: (1) the value of fat and skim established by the butter and nonfat solids market, and (2) the price could be determined from Grade A jobbing cream. With the first method, from an operational standpoint the net values of milk could be determined from yields of butter and powder valued at net prices. There are two such cream markets in Calif. R. W. Hunt

729. Process for canning whole fresh milk. J. C. MOERI (assignor to Farm Fresh Milk Corp.). U. S. Patent 2,642,363. 5 claims. June 16, 1953. Official Gaz. U. S. Pat. Office, **671**, 3: 836. 1953.

Whole milk is sterilized in cans as follows: preheating, homogenizing at 2 800 p.s.i., and filling into sterilized cans, all at approximately 163° F.; heating the sealed cans to 240° F. in a period of 10 min.; holding at 240° F. for 14 min.; cooling to about 100° F. in 10 min., and finally cooling to about 50° F. in an additional 15 min. R. Whitaker

730. Progress toward uniformity in milk ordinances. C. J. BABCOCK, Dairy Branch P.M.A., U.S.D.A. Milk Plant Monthly, **42**, 6: 36-38. 1953.

The greatest progress toward uniform milk ordinances is the wide recognition that uniformity is necessary if we are to have the best possible milk supply for all the people, and that uniformity is essential for the free flow of milk needed to attain the per capita consumption of milk necessary for the health of the nation. There is too little activity by the

individual states toward uniformity on a state basis. Many have failed to recognize that without free intra-state shipments of milk we cannot hope to have free inter-state shipments.
C. J. Babcock

731. Cold milk separation. W. M. Roberts, L. F. Blanton, and G. P. Marley, N. Carolina State Coll. Milk Plant Monthly, **42**, 6: 19-22. 1953.

The separation of cold milk eliminates necessity for preheating and cooling equipment, requires less labor and utilities, and may improve the quality of dairy products by eliminating the forewarming treatment which often results in damage to the flavor and keeping quality. Data are presented to show that separating milk at 42° F. produced cream with higher viscosity than milk separated at 90° F., regardless of whether the separation occurred prior to or following pasteurization. The most viscous cream was obtained by separating cold raw milk before pasteurization. Immediate cooling to 40° F. resulted in cream with slightly higher viscosity than cooling quickly to 50° F. followed by slow cooling to 40° F. The temperature of separation of raw milk and the rate of cooling did not have an important effect on the whipping properties of the cream. Pasteurization of the milk prior to separation did not decrease the whipping ability of the cream. Repasteurization of the cream by the HTST method did not alter the viscosity and whipping ability appreciably from that of the cream obtained from pasteurized milk. Pasteurization of cream by vacreation reduced the viscosity and whipping ability more than HTST pasteurization. When HTST pasteurization was used, cream from the raw milk appeared to be the most resistant to feathering. Serum separation was prevented in 20% cream pasteurized by HTST by standardization with homogenized milk.
C. J. Babcock

732. Sanitary milk carton. O. Komeo. U. S. Patent 2,643,815. 2 claims. June 30, 1953. Official Gaz. U. S. Pat. Office, **671**, 5: 1402. 1953.

A waxed paper milk bottle having a flat bottom and a flanged truncated pyramidal shaped top, having a hinged cover which extends over the entire top to protect the pouring lip.
R. Whitaker

733. Milk bottle case and bottom frame therefor. J. F. Maurer. U. S. Patent 2,643,018. 1 claim. June 23, 1953. Official Gaz. U. S. Pat. Office, **671**, 4: 1105. 1953.

A wooden milk bottle case with a bottom and partitions made of wire.
R. Whitaker

734. Cream separator and cap. E. Scheemaeker. U. S. Patent 2,645,349. 1 claim. July 14, 1953. Official Gaz. U. S. Pat. Office, **672**, 2: 472. 1953.

A device for closing the upper chamber of a cream top bottle for completely removing the cream.
R. Whitaker

735. Bottle. R. A. Blunt. U. S. Patent 2,644,599. 5 claims. July 7, 1953. Official Gaz. U. S. Pat. Office, **672**, 1: 191. 1953.

A design for a cream top type of glass milk bottle.
R. Whitaker

NUTRITIVE VALUE OF DAIRY PRODUCTS

736. Heat processing and the nutritive value of milk and milk products. L. J. Schroeder, M. Iacobellis, and A. H. Smith. J. Nutrition, **49**, 4: 549-561. 1953.

Autoclaving whole milk at 10 or 15 lb. pressure for 15 or 30 min. had no detrimental effect on the protein as measured by digestibility, biological value or nutritive index when fed to dogs. The digestibility and biological value of milk protein do not change when milk is kept in frozen storage for 15 mo. Commercial processing used in the preparation of evaporated milk and milk powder does not decrease the nutritive value of the milk proteins.
O. T. Stallcup

737. The effect of cystine and methionine, added separately or together, on the biological value of the proteins of dried skimmilk. Kathleen M. Henry and S. K. Kon, Univ. Reading, Engl. Brit. J. Nutrition, 7: 29-33. 1953. (Chem. Abstr., 47: 6007h. 1953.)
S. Patton

738. Free amino acids in the serum of infants after feeding with homogenized and non-homogenized milk. Wofgang Reinke, Städt. Krankenhaus Süd, Lübeck, Ger. Z. Kinderheilk, 72: 347-350. 1953. (Chem. Abstr., 47: 6008h. 1953.)
S. Patton

PHYSIOLOGY AND ENDOCRINOLOGY

739. The influence of alpha-tocopherol upon the utilization of carotene and vitamin A. J. W. Herbert and A. F. Morgan. J. Nutrition, **50**, 2: 175-190. 1953.

The addition of 0.5 mg. alpha-tocopherol daily to the diet of rats produced a significant increase in liver vitamin A when the total carotene in oil intake was from 1,200 to 2,400 Ug., or the daily intake from 87 to 174 Ug. for 14 d. Above and below these levels no effect was found. Carotene in aqueous solution (Tween 40) produced larger amounts of liver vitamin A than carotene in the oily medium, but tocopherol was ineffective or depressant under these conditions.
O. T. Stallcup

740. A quantitative study of rumen synthesis in the bovine on natural and purified rations, III. Riboflavin, pantothenic acid and niacin.

I. P. AGRAWALA, C. F. HUFFMAN, R. W. LUECKE, and C. W. DUNCAN. J. Nutrition, **49**, 4: 631-638. 1953.

The authors present evidence indicating that the microorganisms in the rumen of a fistulated calf on a purified ration containing urea as the only essential source of nitrogen were able to synthesize as much riboflavin and niacin but only 50% as much pantothenic acid, within 3 hr. after feeding as was found in the rumen of a calf fed a natural ration. The increased amounts of these B vitamins found in the rumen of the calf on the natural ration were approximately equivalent to the amounts ingested in the feed. O. T. Stalleup

741. Determination of endogenous fecal calcium in cattle by a simple isotope dilution method. W. J. VISEK, R. A. MONROE, E. W. SWANSON, and C. L. COMAR. J. Nutrition, **50**, 1: 23-33. 1953.

The authors present a method for estimating endogenous fecal calcium directly in normal cattle by means of 10 daily intravenous injections of radiocalcium, comparison of the specific activities of blood and feces taken on the 4th to 8th day past-injection, and routine determination of calcium balance. In trials using this method the endogenous fecal calcium was not significantly changed when the dietary calcium was varied from normal to a high level but on a low calcium diet a decrease in endogenous fecal calcium was indicated.

O. T. Stalleup

742. Studies on carotenoid metabolism. XIII. The carotenoid composition of the blood, liver, and ovaries of the rat, ewe, cow and frog. J. GANGULY, J. W. MEHL, and H. J. DEUEL, JR. J. Nutrition, **50**, 1: 73-83. 1953.

The small amount of carotenoid present in the liver of the rat after the oral administration of β-carotene was shown to be all trans-β-carotene. Traces of carotene and of carotenols were present in the liver of the ewe. No carotenoids were found in the blood or ovaries. Large quantities of β-carotene were present in the blood, liver, and ovaries of the cow, while small but definite amounts of carotenols also were present. Definite amounts of β-carotene and carotenols occurred in both the liver and ovaries of the frog but none could be detected in the blood. The authors suggest that the variation in absorption and deposition of the carotenoids in the tissues as related to the species of the animal may be associated with the presence or absence of appropriate protein receptors in the blood and tissues of these animals. O. T. Stalleup

743. Influence of fats and fatty acids on the capillaries. J. KRAMAR and V. E. LEVINE. J. Nutrition, **50**, 2: 149-160. 1953.

The authors observed that a fat-deficient diet, lacking in essential fatty acids, may cause the skin capillaries of immature albino rats to become more fragile and more permeable. The drop in capillary resistance occured simultaneously with the fattening of the weight curve, but before the characteristic manifestations of fat deficiency had developed. The low capillary resistance of fat deficient rats was promptly restored when linseed oil, cottonseed oil or pure linoleic acid were added to the diet.

O. T. Stalleup

744. Effects of vitamin B_{12} on the utilization of carotene and vitamin A by the rat. E. G. HIGH and S. S. WILSON. J. Nutrition, **50**, 2: 203-212. 1953.

The intramuscular injection of vitamin B_{12} with or without sulfasuxidine in the diet increased both growth and tissue deposition of vitamin A from carotene fed. An APF supplement also was effective in increasing the utilization of carotene for storage of vitamin A. Vitamin B_{12} was ineffective with regard to the utilization of preformed vitamin A for storage The significance of these observations is discussed in regard to a possible mode of action of B_{12} on carotene and vitamin A metabolism.

O. T. Stalleup

745. Der Gehalt der Kuhmilch an Tocopherol (Vitamin E) im Verlavf der Lactation. (Tocopherol [vitamin E] content of cows' milk during the course of lactation). English summary. F. KIEFERLE, A. SEUSS, and E. SCHRAUDY. Die Milchwissenschaft, **8**, 2: 57-62. 1953.

Samples of cows' milk were examined for the concentration of vitamin E by the method of Emmerie and Engel. The tocopherol was allowed to react with a_1, a, dipyridyl ferric chloride to form a red complex and the density was measured in a Pulfrich photometer.

Mixed herd winter milk (barn feeding) ranged in tocopherol content from 60 to 105 γ% (ave. 87 γ%), and was equal to 17 to 38 γ/g. of fat (ave. 24γ/g).

Examination of milk of several individual animals showed that higher concentrations of tocopherol were present in milk during oestrum than during in between intervals: e.g. average tocopherol content of two oestrum periods was 112 γ% and 30 γ/g. fat, whereas the average of in between period (26 d.) was 75 γ% and 21 γ/g. fat respectively.

Slight differences in tocopherol content in the milk of individual quarters of the same animal were observed; the higher tocopherol content was not associated necessarily with higher fat content in milk.

Successive portions of milk from the same quarter obtained in one milking showed increasing tocopherol content in consecutive portions. However, the tocopherol content per g. fat was highest in the first drawn portion.

Examination of milk from cows at various stages of lactation showed that colostrum milk

was highest in tocopherols with 300 γ% and above (over 70 γ/g. fat); the values dropped rapidly during the first two d. of lactation and showed a further slow decline as lactation progressed.

The above observations lead the authors to conclude that the tocopherol content of cows' milk is controlled by sexual-hormonal reproductive cycle rather than by breed, age, season of the year and feed. I. Peters

746. Biochemical evidence of nutritional status. O. H. LOWRY, Dept. of Pharmacology, Washington Univ. Med. School, St. Louis, Mo. Physiol. Rev., **32**, 4: 431-448. 1952.

The review cited the use of blood, urine, biopsy samples, etc., in obtaining biochemical evidence to indicate the nutritional state of an animal. The biochemical possibilities were illustrated with three of the more completely studied nutrients (ascorbic acid, thiamine, and riboflavin). The review further stressed the significance of tissue concentration of a nutrient and indicated how the tissue concentration might be related to performance and how tissue levels were interrelated with such factors as intake, excretion, and blood levels. 65 references cited. E. G. Moody

747. Fundamental stimulus for erythropoiesis. W. C. GRANT and W. S. ROOT, Dept. of Physiology, Coll. of Physicians and Surgeons, Columbia Univ., New York. Physiol. Rev., **32**, 4:449-498. 1952.

The review concerned itself with factors which restore a reduced red cell volume and which are capable also of producing hypertrophy of the red cell mass of an organism in nutritional balance and in hematological equilibrium with its environment. 639 references were cited. E. G. Moody

748. Cobalt, copper, and molybdenum in the nutrition of animals and plants. H. R. MARSTON, Commonwealth Scientific and Industrial Organization, Univ. of Adelaide, So. Australia. Physiol. Rev., **32**, 1: 66-121. 1952.

The review covers research pertaining to the need and utilization of cobalt, copper, and molybdenum by animals and plants with special consideration given to ruminants. Among other topics, the relation of cobalt to vitamin B_{12} was discussed, as was also the relationship of copper in iron metabolism and the influence of molybdenum on copper metabolism. 828 references were cited. E. G. Moody

749. Water metabolism of desert mammals. KNUT SCHMIDT-NIELSEN and BODIL SCHMIDT-NIELSEN, Kettering Laboratory, Univ. of Cincinnati, Ohio. Physiol. Rev., **32**, 2: 135-166. 1952.

The water metabolism of cattle, as well as several other mammals, was discussed. 168 references were cited. E. G. Moody

750. The nutrition of the young Ayrshire calf. XII. Factors affecting the tocopherol reserves, muscle composition, and muscle histology of 4-day-old calves. K. L. BLAXTER, F. BROWN, and A. M. MACDONALD, Hannah Dairy Research Inst., Kirkhill, Ayr, Scot. Brit. J. Nutrition, 7: 105-123. 1953. (Chem. Abstr., 47: 6510d. 1953.) S. Patton

751. Starvation ketosis in the ruminant. A. ROBERTSON and CHRISTIAN THIN, Univ. Edinburgh, Scot. Brit. J. Nutrition, 7: 181-195. 1953. (Chem. Abstr., 47: 6510h. 1953.) S. Patton

752. Effects of oxytocin and adrenalin on uterine motility in the bovine. R. L. HAYS and N. L. VANDEMARK, Univ. of Ill., Urbana. Am. J. Physiol., 172: 557-560. 1953. (Chem. Abstr., 47: 6558d. 1953.) S. Patton

753. Nutritional value of plant materials. VIII. Lactation failure of rats fed diets containing purified soybean proteins. M. O. SCHULTZE, Univ. of Minnesota, St. Paul. J. Nutrition, 49: 231-243. 1953. (Chem. Abstr., 47: 6003d. 1953.) S. Patton

754. The nutrition of the young Ayrshire calf. XI. The toxicity of cod-liver oil. K. L. BLAXTER, W. A. WOOD, and A. M. MACDONALD, Hannah Dairy Research Inst., Kirkhill, Ayr, Scot. Brit. J. Nutrition, 7: 34-50. 1953. (Chem. Abstr., 47: 6008a. 1953.) S. Patton

755. Mobility of skeletal phosphorus in a mature dairy cow as determined with radioactive phosphorus. A. L. BLACK, M. KLEIBER, A. H. SMITH, and N. P. RALSTON, Univ. of Calif. Agr. Coll., Davis. Proc. Soc. Exptl. Biol. Med., 82: 248-252. 1953. (Chem. Abstr., 47: 6010b. 1953.) S. Patton

SANITATION AND CLEANSING

756. Sanitizing agents in dairy use. W. S. MUELLER *et al.*, Mass. Agr. Expt. Sta., Amherst. Bull. 467, 41-42. 1953.

The design of teat cup liners influenced the amount of quaternary carried into the milk, but the differences were too slight to have any practical significance.

A search was made for an analytical method of determining quaternary ammonium compounds. It was found that as little as 5 p.p.m. of the alkyl-dimethylbenzyl type in aqueous solution could be detected by diazotization followed by coupling of the amine with eosin dye. The application of this procedure to milk analysis is limited because of the interfering action of tryptophane and probably other amino acids.

The loss of calcium hypochlorite, sodium hypochlorite and chloramine at 212° F. was 26, 25 and 6% respectively in solutions of 100 p.p.m. This loss was less than expected.

A laboratory device for ultraviolet treatment of liquids was investigated, and found to be less effective than present pasteurization techniques. R. W. Hunt

757. Undersoegelser over septictankbehandling af mejerispildevand (Analysis of septic-tank treatment of dairy waste disposal). (English summary). P. Roenkilde Poulsen, C. Lind, and K. P. Andersen, State Expt. Sta. Creamery, Hilleroed, Denmark. Bull., 79: 38-47. 1953.

The breakdown of dairy waste was followed in a septic tank consisting of three 5-l flasks. The rate of flow was 5-l per 24 hr. The waste was made by diluting whey and skimmilk to 5 and 2%. The major part of the lactose was fermented in the first flask and pH dropped from about 7.3 to about 5.6. In flasks 2 and 3 pH increased about 0.1 of a unit due to breakdown of protein to NH_3. An average of 70% of the protein in skimmilk was broken down whereas in 2% whey waste 52% and in 5% whey waste only 19% was broken down. In the latter case pH dropped from the initial 7.3 to 4.6.

The oxygen demand, using $KMnO_4$, was reduced to 25% after 3 d. The B.O.D. after 5 d. almost always was higher than the initial. This is explained by the increased availability of the treated material to biological oxidation.

Before treatment of the waste 12-37% of the carbon was in ether-soluble form; after treatment the content was 70-76%. From 9-35% of the carbon escaped as CO_2 and CH_4 during the fermentation. T. Kristoffersen

758. Stainless equipment protection. A. H. Wakeman, The Creamery Package Manufacturing Co., Fort Atkinson, Wis. Milk Plant Monthly, **42**, 6: 27-29. 1953.

Stainless steel is not an indestructible metal. The metal normally is protected by an invisible oxide film. If this film (also known as passivity) is removed and not allowed to reform, corrosion takes place. Passivity may be destroyed by the adhering of materials to the surface, improper grounding, and electrolytic action between two dissimilar metals. Pitting also may be caused by chemical action, especially by hypochlorite, chlorine and chloride solutions.

Methods for cleaning and sterilizing stainless equipment are given. The following don'ts are presented for handling stainless equipment: (1) Don't use steel wool. (2) Don't use a water supply having iron, salt, or sulphur content, (3) Don't allow chemical sterilizers, alkalies or cleansers to remain on surface over 30 minutes, (4) Don't allow foreign matter to adhere to equipment, (5) Don't allow rubber protective items which are fitted over stainless steel surface to remain on such surfaces for any length of time, (6) Don't allow wrenches, sanitary fittings or other dissimilar metal parts to lie on stainless steel surfaces which remain wet, (7) Don't close the covers of a tank after steaming unless a vent is provided to avoid creating a vacuum which might result in collapse of the tank, (8) Don't apply air or steam pressure unless equipment is specifically designed for such pressures. C. J. Babcock

759. House fly control in New York State dairy barns. W. J. Goodwin and H. H. Schwardt. J. Econ. Entomol., **46**, 2: 299-301. 1953.

During 1951 and '52 control trials with 9 insecticidal formulations were made against house flies in 172 dairy barns. Treatment methods were termed overall, spot, strip, or vaporizer. Materials used were lindane, dieldrin, DDT, DDT plus K3936, lindane plus Re 1901, dilan, and methoxychlor plus lindane. None of these gave effective control in 1952 overall and spot treatments. Strip treatments, done only with dieldrin, were effective during 2 yr. where dieldrin had not been previously used, but resistance developed during the third yr. However, the strip method is not presently recommended. Sanitation is very important in dairy barn fly control. E. H. Fisher

760. Failure of residual insecticides to control house flies. E. J. Hansens, Rutgers Univ., New Brunswick, N. J. J. Econ. Entomol., **46**, 2: 246-248. 1953.

During 1949 thru 1951, dairy barns in New Jersey were sprayed 1 to 3 times for housefly control with either lindane, methoxychlor, chlordane, or dieldrin.

In 1952 none of these insecticides gave good control in the same barns, and the best alternate material of the group also failed. Thirty-two lb. of 25% wettable dilan per 100 gal. of spray gave 3 wk. of control in the one barn where tested. Space sprays were ultimately recommended in all these barns.

Laboratory tests with house flies from these barns also disclosed a high degree of fly resistance to the original test insecticides. E. H. Fisher

761. Development and loss of insecticide resistance in the house fly. D. Pimentel, H. H. Schwardt, and J. E. Dewey, Cornell Univ., Ithaca, N. Y. J. Econ. Entomol., **46**, 2: 295-298. 1953.

Laboratory tests with selected strains of house flies in New York showed the development and loss of resistance to certain insecticides. Resistance to DDT and lindane developed rapidly, while with parathion it was much slower, and with dieldrin it was within the range of these other materials. There was an apparent limit to which flies could resist a specific insecticide.

Selective exposure and breeding of one strain of flies showed that by the twentieth generation, 70 to 80% of the flies survived exposure for

2 wk. on a deposit of 4000 mg. DDT per sq. ft. of treated surface. Resistance did not increase during the next 6 generations. Flies from the twentieth generation of this strain were reared thru 22 generations without exposure to DDT, at which time DDT resistance was lost.

There was no difference between a DDT-resistant and non-resistant strain in rate of developing high resistance to lindane. When DDT-resistant flies were selectively bred to become resistant to lindane, dieldrin, or parathion, the DDT-resistance increased in most cases and decreased in none.

Ten to 20 generations of non-exposure of DDT-resistant flies to insecticide caused a loss of resistance. Similar results occurred with lindane. E. H. Fisher

762. Three new insecticides for housefly control in barns. E. J. Hansens, N. J. Agr. Expt. Sta., New Brunswick, and C. E. Bartley, Geigy Co., Inc., Bayonne, N. J. J. Econ. Entomol., **46**, 2: 372-374. 1953.

Housefly control in dairy barns, using pyrolan, pyramat, and diazinon, was studied. Each material was combined individually with methoxychlor, and the latter two also were used alone. These 3 new materials possess strong anticholinesterase action.

Diazinon was best. It gave control up to 10 wk. against non-resistant flies and for 4 wk. against resistant ones. Pyramat may be useful as a quick knockdown material, combined with residual insecticides. E. H. Fisher

763. Forsoeg med udsproejtning af mejerispildevand (Experiments with sprinkling of dairy waste). (English summary). P. Roenkilde Poulsen, H. Skov Rasmussen and K. P. Andersen, State Expt. Sta. Creamery, Hilleroed, Denmark. Bull., 79: 9-37. 1953.

During May-Nov. 1945, 1946, 1947 and 1948 dairy waste was disposed of by sprinkling on pasture land. The amount of waste corresponded to 1/3 of the normal rainfall. The yield of clover and grasses was better than doubled by weight per m^2 of the sprinkled areas. The increase in nutritional value however, was only about 80% because of a lower dry matter content.

In the early part of the summer the cows avoided the grass on the sprinkled areas for about 2 wk. after sprinkling. This time was reduced to 4-5 d. during midsummer and fall. Analysis of the soil showed no significant changes over the 4 yr. period.

The general condition of the cows was not changed. Bang's disease did not appear to be spread. Over a 7 yr. period the no. of cases of Bang's disease steadily decreased among cows producing milk to 8 different creameries. The cows supplying 2 of the creameries remained free of Bang's disease throughout the entire period. T. Kristoffersen

764. Can washer selection for the dairy plant. C. W. Hall. Mich. Agr. Expt. Sta. Bull., 35: 461-466. 1953.

There is a definite relationship between the size of can washer and the rate at which milk cans can be dumped. Methods are outlined, and illustrated by example, for determining the most economical size of straight-away and rotary can washers. A 10-CPM washer is the proper size for a typical one-man receiving room where an average of 5 or fewer cans are received daily from one producer. The rotary can washer is more economical than the straight-away washer for plants receiving up to 10,000 lb. of milk per day. R. Hunt

765. Can cover inserting device. H. D. Lathrop (assignor to The Lathrop-Paulson Co.). U. S. Patent 2,645,400. 3 claims. July 14, 1953. Official Gaz. U. S. Pat. Office, **672**, 2: 487. 1953.

Structural details are given for an attachment to a can washer for automatically placing the can lid upside down beneath the inverted clean can, lowering the can down onto the lid and then upending the can and lid for discharge from the washer. R. Whitaker

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